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(57) Abstract

The present invention relates to the cloning and high level expression of novel truncated cellulase proteins or derivatives thereof in the filamentous fungus Trichoderma longibrachiatum. Further aspects of the present invention relate to fungal transformants that express the novel truncated cellulases and derivatives, and expression vectors comprising the DNA gene fragments or variants thereof that code for the truncated cellulases derived from Trichoderma longibrachiatum using genetic engineering techniques.

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NOVEL CELLULASE ENZYMES AND SYSTEMS FOR THEIR EXPRESSION

Field of the Invention

The present invention relates to a process for producing high levels of novel truncated cellulase proteins in the filamentous fungus Trichoderma longibrachiatum; to fungal transformants produced from Trichoderma longibrachiatum by genetic engineering techniques; and to novel cellulase proteins produced by such transformants.

Background of the Invention

Cellulases are enzymes which hydrolyze cellulose (β-1,4-D-glucan linkages) and produce as primary products glucose, cellobiose, cellooligosaccharides, and the like. Cellulases are produced by a number of microorganisms and comprise several different enzyme classifications including those identified as exo-cellobiohydrolases (CBH), endoglucanases (EG) and β-glucosidases (BG) (Schulein, M, 1988 Methods in Enzymology 160: 235-242). Moreover, the enzymes within these classifications can be separated into individual components. For example, the cellulase produced by the filamentous fungus, Trichoderma longibrachiatum, hereafter T.longibrachiatum, consists of at least two CBH components, i.e., CBHI and CBHII, and at least four EG components, i.e., EGI, EGII, EGIII and EGV (Saloheimo, A. et al 1993 in Proceedings of the second TRICEL symposium on Trichoderma reesei Cellulases and Other Hydrolases, Espoo, Finland, ed by P. Suominen & T. Reinikainen. Foundation for Biotechnical and Industrial Fermentation Research 8: 139-146) components, and at least one β-glucosidase. The genes encoding these components are namely cbh1, cbh2, egl1, egl2, egl3, and egl5 respectively.

The complete cellulase system comprising CBH, EG and BG components synergistically act to convert crystalline cellulose to glucose. The two exo-cellobiohyrolases and the four presently known endoglucanases act together to hydrolyze cellulose to small cello-oligosaccharides. The

oligosaccharides (mainly cellobioses) are subsequently hydrolyzed to glucose by a major β-glucosidase (with possible additional hydrolysis from minor β-glucosidase components).

Protein analysis of the cellobiohydrolases (CBHI and CBHII) and major endoglucanases (EGI and EGII) of T. longibrachiatum have shown that a bifunctional organization exists in the form of a catalytic core domain and a smaller cellulose binding domain separated by a linker or flexible hinge stretch of amino acids rich in proline and hydroxyamino acids. Genes for the two cellobiohydrolases, CBHI and CBHII (Shoemaker, S et al 1983 Bio/Technology 1, 691-696, Teeri, T et al 1983, Bio/Technology 1, 696-699 and Teeri, T. et al, 1987, Gene 51, 43-52) and two major endoglucansases, EGI and EGII (Penttila, M. et al 1986, Gene 45, 253-263, Van Arsdell, J.N/ et al 1987 Bio/Technology 5, 60-64 and Saloheimo, M. et al 1988, Gene 63, 11-21) have been isolated from T. longibrachiatum and the protein domain structure has been confirmed.

A similar bifunctional organization of cellulase enzymes is found in bacterial cellulases. The cellulose binding domain (CBD) and catalytic core of Cellulomonas fimi endoglucanase A (C. fimi Cen A) has been studied extensively (Ong E. et al 1989, Trends Biotechnol. 7:239-243, Pilz et al 1990, Biochem J. 271:277-280 and Warren et al 1987, Proteins 1:335-341). Gene fragments encoding the CBD and the CBD with the linker have been cloned, expressed in E. coli and shown to possess novel activities on cellulose fibers (Gilkes, N.R. et al 1991, Microbiol Rev. 55:305-315 and Din, N et al 1991, Bio/Technology 9:1096-1099). For example, isolated CBD from C. fimi Cen A genetically expressed in E. coli disrupts the structure of cellulose fibers and releases small particles but have no detectable hydrolytic activity. CBD further possess a wide application in protein purification and enzyme immobilization. On the other hand, the catalytic domain of C. fimi Cen A isolated from protease cleaved cellulase does not disrupt the fibril structure of cellulose and instead smooths the surface of the fiber.

These novel activities have potential uses in textile, food and animal feed, detergents and the pulp and paper industries. However, for industrial application, highly efficient expression systems must be procured that produce higher yields of truncated cellulase proteins than are currently available to be of any commercial value. For example, Trichoderma longibrachiatum CBHI core domains have been separated proteolytically and purified but only milligram quantities are isolated by this biochemical procedure (Offord D., et al 1991, Applied Biochem. and Biotech. **28/29**:377-386). Similar studies were done in an analysis of the core and binding domains of CBHI, CBHII, EGI and EGII isolated from T. longibrachiatum after biochemical proteolysis, however, only enough protein was recovered for structural and functional analysis (Tomme, P et al, 1988, Eur.J. Biochem **170**:575-581 and Ajo, S, 1991 FEBS **291**:45-49).

In order to obtain strains which express higher levels of truncated cellulase proteins than previously realized, applicants chose T. longibrachiatum as the microorganism most preferred for expression since it is well known for its capacity to secrete whole cellulases in large quantities. Thus, applicants set out to genetically engineer strains of the above filamentous fungus to express high levels of bioengineered novel protein truncated cellulases.

It remained unknown before Applicants invention whether the DNA encoding truncated cellulase binding and core domain proteins could be transformed into Trichoderma in such a manner as to overexpress novel truncated cellulase genes into functional proteins without deterioration in the host cell and obtained secretion to facilitate identification and purification of the engineered product. Recently, Nakari and Penttila have shown that it is possible to genetically engineer a Trichoderma host to express a truncated form of the Trichoderma EGI cellulase, specifically the catalytic core domain, however the level of expression of EGI core domain was low (Nakari, T. et al, Abstract P1/63 1st European Conference on Fungal Genetics, Nottingham, England, August 20-23, 1992).

Moreover, it was unknown whether a Trichoderma cellobiohydrolase catalytic core domain or any Trichoderma cellobiohydrolase or endoglucanase cellulose binding domain could be produced by recombinant genetic methods.

Accordingly, it is an object of the present invention to introduce DNA gene fragments into strains of the fungus, Trichoderma longibrachiatum to produce transformant strains that express high levels of novel truncated protein (grams/liter level) engineered cellulases from the binding and core domains of Trichoderma cellulases. The truncated proteins are correctly processed and secreted extracellularly in an active form. The present invention further relates to the novel truncated proteins isolated from these transformants.

Summary of the Invention

Methods involving recombinant DNA technology and compositions are provided for the production and isolation of novel truncated cellulase proteins, derivatives thereof or covalently linked truncated cellulase domain derivatives derived from the filamentous fungus, Trichoderma sp. The truncated cellulase comprises at least a core or binding domain of a cellobiohydrolases or endoglucanase from the species Trichoderma. Derivatives of truncated cellulases include substitutions, deletions, or additions of one or more amino acids at various sites throughout the core or binding domain of the novel truncated cellulase whereby either the cellulose binding or cellulase catalytic core activity is retained. Covalently linked truncated cellulase domain derivatives comprise truncated cellulases or derivatives thereof that are further attached to each other, and/or enzymes, or domains and/or proteins, and/or chemicals heterologous or homologous to Trichoderma sp.

The present invention also includes the preparation of novel truncated cellulases, derivatives and covalently linked truncated cellulase domain derivatives by transforming into a host cell a DNA construct comprising a DNA fragment or variant

thereof encoding the above novel cellulase(s) functionally attached to regulatory sequences that permit the transcription and translation of the structural gene and growing the host cell to express the truncated gene of interest.

The present invention further includes DNA fragments and variants thereof encoding novel truncated cellulases, derivatives and covalently linked truncated cellulase domain derivatives. The present invention also encompasses expression vectors comprising the above DNA fragments or variants thereof and Trichoderma host cells transformed with the above expression vectors.

Brief Detailed Description of the Drawings

Figure 1 depicts the genomic DNA and amino acid sequence of CBHI derived from Trichoderma longibrachiatum. The signal sequence begins at base pair 210 and ends at base pair 260 (Seq ID No. 25). The catalytic core domain begins at base pair 261 through base pair 671 of the first exon, base pair 739 through base pair 1434 of the second exon, and base pair 1498 through base pair 713 of the third exon (Seq ID No. 9). The linker sequence begins at base pair 714 and ends at base pair 1785 (Seq ID No. 17). The cellulase binding domain begins at base pair 1786 and ends at base pair 1888 (Seq ID No. 1). Seq ID Nos. 26, 10, 18 and 2 represent the amino acid sequence of the CBHI signal sequence, catalytic core domain, linker region and binding domain, respectively.

Figure 2 depicts the genomic DNA and amino acid sequence of CBHII derived from Trichoderma longibrachiatum. The signal sequence begins at base pair 614 and ends at base pair 685 (Seq ID No. 27). The cellulose binding domain begins at base pair 686 through base pair 707 of exon one, and base pair 755 through base pair 851 of exon two (Seq ID No. 3). The linker sequence begins at base pair 852 and ends at base pair 980 (Seq ID No. 19). The catalytic core begins at base pair 981 through base pair 1141 of exon two, base pair 1199 through base pair 1445 of exon three and base pair 1536 through base pair 2221 of exon four (Seq ID No. 11). Seq ID Nos. 28, 4, 20

and 12 represent the amino acid sequence of the CBHII signal sequence, binding domain, linker region and catalytic core domain, respectively.

Figure 3 depicts the genomic DNA and amino acid sequence of EGI. The signal sequence begins at base pair 113 and ends at base pair 178 (Seq ID No. 29). The catalytic core domain begins at base pair 179 through 882 of exon one, and base pair 963 through base pair 1379 of the second exon (Seq ID No. 13). The linker region begins at base pair 1380 and ends at base pair 1460 (Seq ID No. 21). The cellulose binding domain begins at base pair 1461 and ends at base pair 1616 (Seq ID No. 5). Seq ID Nos. 30, 14, 22 and 6 represent the amino acid sequence of EGI signal sequence, catalytic core domain, linker region and binding domain, respectively.

Figure 4 depicts the genomic DNA and amino acid sequence of EGII. The signal sequence begins at base pair 262 and ends at base pair 324 (Seq ID No. 31). The cellulose binding domain begins at base pair 325 and ends at base pair 432 (Seq ID No. 7). The linker region begins at base pair 433 and ends at base pair 534 (Seq No. 23). The catalytic core domain begins at base pair 535 through base pair 590 in exon one, and base pair 765 through base pair 1689 in exon two (Seq ID No. 15). Seq ID Nos. 32, 8, 24 and 16 represent the amino acid sequence of EGII signal sequence, binding domain, linker region and catalytic core domain, respectively.

Figure 5 depicts the genomic DNA and amino acid sequence of EGIII. The signal sequence begins at base pair 151 and ends at base pair 198 (Seq ID No. 36). The catalytic core domain begins at base pair 199 through base pair 557 in exon one, base pair 613 through base pair 833 in exon two and base pair 900 through base pair 973 in exon three (Seq ID No. 33). Seq ID Nos. 36 and 34 represent the amino acid sequence of EGIII signal sequence and catalytic core domain, respectively.

Figure 6 illustrates the construction of EGI core domain expression vector (Seq ID No. 37).

Figure 7 depicts the construction of the expression plasmid pTEX (Seq ID Nos. 39-41).

Figure 8 is an illustration of the construction of CBHI core domain expression vector (Seq ID No. 38).

Figure 9 is an illustration of the construction of CBHII cellulase binding domain expression vector (Seq ID Nos. 42 and 43).

Detailed Description

As noted above, the present invention generally relates to the cloning and expression of novel truncated cellulase proteins at high levels in the filamentous fungus, T. longibrachiatum. Further aspects of the present invention will be discussed in further detail following a definition of the terms employed herein.

The term "Trichoderma" or "Trichoderma sp." refers to any fungal strains which have previously been classified as Trichoderma or which are currently classified as Trichoderma. Preferably the species are Trichoderma longibrachiatum, Trichoderma reesei or Trichoderma viride.

The terms "cellulolytic enzymes" or "cellulase enzymes" refer to fungal exoglucanases or exocellobiohydrolases (CBH), endoglucanses (EG) and β-glucosidases (BG). These three different types of cellulase enzymes act synergistically to convert crystalline cellulose to glucose. Analysis of the genes coding for CBHI, CBHII and EGI and EGII show a domain structure comprising a catalytic core region (CCD), a hinge or linker region (used interchangeably herein) and cellulose binding region (CBD).

The term "truncated cellulases", as used herein, refers to the core or binding domains of the cellobiohydrolases and endoglucanases, for example, EGI, EGII, EGIII, EGV, CBHI and CBHII, or derivatives of either of the truncated cellulase domains.

A "derivative" of the truncated cellulases encompasses the core or binding domains of the cellobiohydrolases, for example, CBHI or CBHII, and the endoglucanases, for example, EGI, EGII, EGIII and EGV from Trichoderma sp, wherein there may be an addition of one or more amino acids to either or

both of the C- and N- terminal ends of the truncated cellulase, a substitution of one or more amino acids at one or more sites throughout the truncated cellulase, a deletion of one or more amino acids within or at either or both ends of the truncated cellulase protein, or an insertion of one or more amino acids at one or more sites in the truncated cellulase protein such that exoglucanase and endoglucanase activities are retained in the derivatized CBH and EG catalytic core truncated proteins and/or the cellulose binding activity is retained in the derivatized CBH and EG binding domain truncated proteins. It is also intended by the term "derivative of a truncated cellulase" to include core or binding domains of the exoglucanase or endoglucanase enzymes that have attached thereto one or more amino acids from the linker region.

A truncated cellulase protein derivative further refers to a protein substantially similar in structure and biological activity to a cellulase core or binding domain which comprises the cellulolytic enzymes found in nature, but which has been engineered to contain a modified amino acid sequence. Thus, provided that the two proteins possess a similar activity, they are considered "derivatives" as that term is used herein even if the primary structure of one protein does not possess the identical amino acid sequence to that found in the other.

The term "cellulase catalytic core domain activity" refers herein to an amino acid sequence of the truncated cellulase comprising the core domain of the cellobiohydrolases and endoglucanases, for example, EGI, EGII, EGIII, EGV, CBHI or CBHII or a derivative thereof that is capable of enzymatically cleaving a cellulosic polymers such as pulp or phosphoric acid swollen cellulose.

The activity of the truncated catalytic core proteins or derivatives thereof as defined herein may be determined by methods well known in the art. (See Wood, T.M. et al in Methods in Enzymology, Vol. 160, Editors: Wood, W.A. and Kellogg, S.T., Academic Press, pp. 87-116, 1988) For example, such activities can be determined by hydrolysis of phosphoric

acid-swollen cellulose and/or soluble oligosaccharides followed by quantification of the reducing sugars released. In this case the soluble sugar products, released by the action of CBH or EG catalytic domains or derivatives thereof, can be detected by HPLC analysis or by use of colorimetric assays for measuring reducing sugars. It is expected that these catalytic domains or derivatives thereof will retain at least 10% of the activity exhibited by the intact enzyme when each is assayed under similar conditions and dosed based on similar amounts of catalytic domain protein.

The term "cellulose binding domain activity" refers herein to an amino acid sequence of the cellulase comprising the binding domain of cellobiohydrolases and endoglucanases, for example, EGI, EGII, CBHI or CHBII or a derivative thereof that non-covalently binds to a polysaccharide such as cellulose. It is believed that cellulose binding domains (CBDs) function independently from the catalytic core of the cellulase enzyme to attach the protein to cellulose.

The performance (or activity) of the truncated binding domain or derivatives thereof as described in the present invention may be determined by cellulose binding assays using a cellulosic substrates such as avicel, pulp or cotton, for example. It is expected that these novel truncated binding domains or derivatives thereof will retain at least 10% of the binding affinity compared to that exhibited by the intact enzyme when each is assayed under similar conditions and dosed based on similar amounts of binding domain protein. The amount of non-bound binding domain may be quantified by direct protein analysis, by chromatographic methods, or possibly by immunological methods.

Other methods well known in the art that measure cellulase catalytic and/or binding activity via the physical or chemical properties of particular treated substrates may also be suitable in the present invention. For example, for methods that measure physical properties of a treated substrate, the substrate is analyzed for modification of shape, texture, surface, or structional properties,

modification of the "wet" ability, e.g. substrates ability to absorb water, or modification of swelling. Other parameters which may determine activity include the measuring of the change in the chemical properties of treated solid substrates. For example, the diffusion properties of dyes or chemicals may be examined after treatment of solid substrate with the truncated cellulase binding protein or derivatives thereof described in the present invention. Appropriate substrates for evaluating activity include Avicel, rayon, pulp fibers, cotton or ramie fibers, paper, kraft or ground wood pulp, for example. (See also Wood, T.M. et al in "Methods in Enzymology", Vol. 160, Editors: Wood, W.A. and Kellogg, S.T., Academic Press, pp. 87-116, 1988)

The term "linker or hinge region" refers to the short peptide region that links together the two distinct functional domains of the fungal cellulases, i.e., the core domain and the binding domain. These domains in T. longibrachiatum cellulases are linked by a peptide rich in Ser Thr and Pro.

A "signal sequence" refers to any sequence of amino acids bound to the N-terminal portion of a protein which facilitates the secretion of the mature form of the protein outside of the cell. This definition of a signal sequence is a functional one. The mature form of the extracellular protein lacks the signal sequence which is cleaved off during the secretion process.

The term "variant" refers to a DNA fragment encoding the CBH or EG core or binding domain that may further contain an addition of one or more nucleotides internally or at the 5′ or 3′ end of the DNA fragment, a deletion of one or more nucleotides internally or at the 5′ or 3′ end of the DNA fragment or a substitution of one or moere nucleotides internally or at the 5′ or 3′ end of the DNA fragment wherein the functional activity of the binding and core domains that encode for a truncated cellulase is retained.

A variant DNA fragment comprising the core or binding domain is further intended to indicate that a linker or hinge DNA sequence or portion thereof may be attached to the core or

binding domain DNA sequence at either the 5′ or 3′ end wherein the functional activity of the encoded truncated binding or core domain protein (derivative) is retained.

The term "host cell" means both the cells and protoplasts created from the cells of Trichoderma sp.

The term "DNA construct or vector" (used interchangeably herein) refers to a vector which comprises one or more DNA fragments or DNA variant fragments encoding any one of the novel truncated cellulases or derivatives described above.

The term "functionally attached to" means that a regulatory region, such as a promoter, terminator, secretion signal or enhancer region is attached to a structural gene and controls the expression of that gene.

The present invention relates to truncated cellulases, derivatives of truncated cellulases and covalently linked truncated cellulase domain derivatives that are prepared by recombinant methods by transforming into a host cell, a DNA construct comprising at least a fragment of DNA encoding a portion or all of the binding or core region of the cellobiohydrolases or endoglucanases, for example, EGI, EGII, EGIII, EGV, CBHI or CBHII functionally attached to a promoter, growing the host cell to express the truncated cellulase, derivative truncated cellulase or covalently linked truncated cellulase domain derivatives of interest and subsequently purifying the truncated cellulase, or derivative thereof to substantial homogeneity.

It is further contemplated by the present invention that one may generate novel derivatives of cellulase enzymes which, for instance, combine a core region derived from a truncated endoglucanase or exocellobiohydrolase of the present invention with a cellulose-binding domain derived from another cellulase enzyme from multiple microbial sources such as fungal and bacterial. Alternatively, it may be possible to combine a core region derived from another cellulase enzyme with a cellulose-binding domains derived from a truncated endoglucanase or exocellobiohydralase of the present invention. In a particular embodiment, the core region may be

derived from a cellulase enzyme which does not in nature comprise a cellulose-binding domain, for example, EGIII (Figure 5 and SEQ ID Nos. 33 and 34), and which is N- or C-terminally extended with a truncated cellulase or derivative thereof comprising a cellulose-binding domain described herein. In this way, it may be possible to construct novel cellulase enzymes with altered cellulose binding properties compared to natural intact cellulases.

In yet another aspect of the present invention, it is contemplated that truncated cellulases or derivatives thereof of the present invention may be further attached to each other and/or to intact proteins and/or enzymes and/or portions thereof, for example, hemicellulases, immunoglobulins, and/or binding or core domains from non Trichoderma cellulases, and/or from non-cellulase enzymes using the recombinant methods described herein to form novel covalently linked truncated cellulase domain derivatives. These covalently linked truncated cellulase domain derivatives constructed in this manner may provide even further benefits over the truncated cellulases or derivatives thereof disclosed in the present invention. It is contemplated that these covalently linked truncated cellulase domain derivatives which contain other enzymes, proteins or portions thereof may exhibit bifunctional activity and/or bifunctional binding.

In yet a further aspect, the present invention relates to a method of producing a truncated cellulase or derivative thereof which method comprises cultivating a host cell as described above under conditions such that production of the truncated cellulase or derivative thereof is effected and recovering the truncated cellulase or derivative from the cells or culture medium.

Highly enriched truncated cellulases are prepared in the present invention by genetically modifying microorganisms described in further detail below. Transformed microorganism cultures are grown to stationary phase, filtered to remove the cells and the remaining supernatant is concentrated by

ultrafiltration to obtain a truncated cellulase or a derivative thereof.

In a particular aspect of the above method, the medium used to cultivate the transformed host cells may be any medium suitable for cellulase production in Trichoderma. The truncated cellulases or derivatives thereof are recovered from the medium by conventional techniques including separations of the cells from the medium by centrifugation, or filtration, precipitation of the proteins in the supernatant or filtrate with salt, for example, ammonium sulphate, followed by chromatography procedures such as ion exchange chromatography, affinity chromatography and the like.

Alternatively, the final protein product may be isolated and purified by binding to a polysaccharide substrate or antibody matrix. The antibodies (polyclonal or monoclonal) may be raised against cellulase core or binding domain peptides, or synthetic peptides may be prepared from portions of the core domain or binding domain and used to raise polyclonal antibodies.

In a general embodiment of the present method, one or more functionally active truncated cellulases or derivatives thereof is expressed in a Trichoderma host cell transformed with a DNA vector comprising one or more DNA fragments or variant fragments encoding truncated cellulases, derivatives thereof or covalently linked truncated cellulase domain derivative proteins. The Trichoderma host cell may or may not have been previously manipulated through genetic engineering to remove any host genes that encode intact cellulases.

In a particular embodiment, truncated cellulases, derivatives thereof or covalently linked truncated cellulase domain derivatives are expressed in transformed Trichoderma cells in which genes have not been deleted therefrom. The truncated proteins listed above are recovered and separated from intact cellulases expressed simultaneously in the host cells by conventional procedures discussed above including sizing chromatography. Confirmation of expression of truncated cellulases or derivatives is determined by SDS

polyacrylamide gel electrophoresis and Western immunoblot analysis to distinguish truncated from intact cellulase proteins.

In a preferred embodiment, the present invention relates to a method for transforming a Trichoderma sp host cell that is missing one or more cellulase activities and treating the cell using recombinant DNA techniques well known in the art with one or more DNA fragments encoding a truncated cellulase, derivative thereof or covalently linked truncated cellulase domain derivatives. It is contemplated that the DNA fragment encoding a derivative truncated cellulase core or binding domain may be altered such as by deletions, insertions or substitutions within the gene to produce a variant DNA that encodes for an active truncated cellulase derivative.

It is further contemplated by the present invention that the DNA fragment or DNA variant fragment encoding the truncated cellulase or derivative may be functionally attached to a fungal promoter sequence, for example, the promoter of the cbh1 or egl1 gene.

Also contemplated by the present invention is manipulation of the Trichoderma sp. strain via transformation such that a DNA fragment encoding a truncated cellulase or derivative thereof is inserted within the genome. It is also contemplated that more than one copy of a truncated cellulase DNA fragment or DNA variant fragment may be recombined into the strain.

A selectable marker must first be chosen so as to enable detection of the transformed fungus. Any selectable marker gene which is expressed in Trichoderma sp. can be used in the present invention so that its presence in the transformants will not materially affect the properties thereof. The selectable marker can be a gene which encodes an assayable product. The selectable marker may be a functional copy of a Trichoderma sp gene which if lacking in the host strain results in the host strain displaying an auxotrophic phenotype.

The host strains used could be derivatives of Trichoderma sp which lack or have a nonfunctional gene or genes corresponding to the selectable marker chosen. For example, if the selectable marker of pyr4 is chosen, then a specific pyr derivative strain is used as a recipient in the transformation procedure. Other examples of selectable markers that can be used in the present invention include the Trichoderma sp. genes equivalent to the Aspergillus nidulans genes argB, trpC, niaD and the like. The corresponding recipient strain must therefore be a derivative strain such as $argB^-$, $trpC^-$, $niaD^-$, and the like.

The strain is derived from a starting host strain which is any Trichoderma sp. strain. However, it is preferable to use a T. longibrachiatum cellulase over-producing strain such as RL-P37, described by Sheir-Neiss et al. in Appl. Microbiol. Biotechnology, 20 (1984) pp. 46-53, since this strain secretes elevated amounts of cellulase enzymes. This strain is then used to produce the derivative strains used in the transformation process.

The derivative strain of Trichoderma sp. can be prepared by a number of techniques known in the art. An example is the production of $pyr4^-$ derivative strains by subjecting the strains to fluoroorotic acid (FOA). The pyr4 gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. Strains with an intact pyr4 gene grow in a medium lacking uridine but are sensitive to fluoroorotic acid. It is possible to select $pyr4^-$ derivative strains which lack a functional orotidine monophosphate decarboxylase enzyme and require uridine for growth by selecting for FOA resistance. Using the FOA selection technique it is also possible to obtain uridine requiring strains which lack a functional orotate pyrophosphoribosyl transferase. It is possible to transform these cells with a functional copy of the gene encoding this enzyme (Berges and Barreau, 1991, Curr. Genet. 19 pp359-365). Since it is easy to select derivative strains using the FOA resistance

technique in the present invention, it is preferable to use the pyr4 gene as a selectable marker.

In a preferred embodiment of the present invention, Trichoderma host cell strains have been deleted of one or more cellulase genes prior to introduction of a DNA construct or plasmid containing the DNA fragment encoding the truncated cellulase protein of interest. It is preferable to express a truncated cellulase, derivative thereof or covalently linked truncated cellulase domain derivatives in a host that is missing one or more cellulase genes in order to simplify the identification and subsequent purification procedures. Any gene from Trichoderma sp. which has been cloned can be deleted such as cbh1, cbh2, egl1, egl3, and the like. The plasmid for gene deletion is selected such that unique restriction enzyme sites are present therein to enable the fragment of homologous Trichoderma sp. DNA to be removed as a single linear piece.

The desired gene that is to be deleted from the transformant is inserted into the plasmid by methods known in the art. The plasmid containing the gene to be deleted or disrupted is then cut at appropriate restriction enzyme site(s), internal to the coding region, the gene coding sequence or part thereof may be removed therefrom and the selectable marker inserted. Flanking DNA sequences from the locus of the gene to be deleted or disrupted, preferably between about 0.5 to 2.0 kb, remain on either side of the selectable marker gene.

A single DNA fragment containing the deletion construct is then isolated from the plasmid and used to transform the appropriate pyr$^-$ Trichoderma host. Transformants are selected based on their ability to express the pyr4 gene product and thus compliment the uridine auxotrophy of the host strain. Southern blot analysis is then carried out on the resultant transformants to identify and confirm a double cross over integration event which replaces part or all of the coding region of the gene to be deleted with the pyr4 selectable markers.

Although specific plasmid vectors are described above, the present invention is not limited to the production of these vectors. Various genes can be deleted and replaced in the Trichoderma sp. strain using the above techniques. Any available selectable markers can be used, as discussed above. Potentially any Trichoderma sp. gene which has been cloned, and thus identified, can be deleted from the genome using the above-described strategy. All of these variations are included within the present invention.

The expression vector of the present invention carrying the inserted DNA fragment or variant DNA fragment encoding the truncated cellulase or derivative thereof of the present invention may be any vector which is capable of replicating autonomously in a given host organism, typically a plasmid. In preferred embodiments two types of expression vectors for obtaining expression of genes or truncations thereof are contemplated. The first contains DNA sequences in which the promoter, gene coding region, and terminator sequence all originate from the gene to be expressed. The gene truncation is obtained by deleting away the undesired DNA sequences (coding for unwanted domains) to leave the domain to be expressed under control of its own transcriptional and translational regulatory sequences. A selectable marker is also contained on the vector allowing the selection for integration into the host of multiple copies of the novel gene sequences.

For example, pEGIΔ3'pyr contains the EGI cellulase core domain under the control of the EGI promoter, terminator, and signal sequences. The 3' end on the EGI coding region containing the cellulose binding domain has been deleted. The plasmid also contains the pyr4 gene for the purpose of selection.

The second type of expression vector is preassembled and contains sequences required for high level transcription and a selectable marker. It is contemplated that the coding region for a gene or part thereof can be inserted into this general purpose expression vector such that it is under the

transcriptional control of the expression cassettes promoter and terminator sequences.

For example, pTEX is such a general purpose expression vector. Genes or part thereof can be inserted downstream of the strong CBHI promoter. The Examples disclosed herein are included in which cellulase catalytic core and binding domains are shown to be expressed using this system.

In the vector, the DNA sequence encoding the truncated cellulase or other novel proteins of the present invention should be operably linked to transcriptional and translational sequences, i.e., a suitable promoter sequence and signal sequence in reading frame to the structural gene. The promoter may be any DNA sequence which shows transcriptional activity in the host cell and may be derived from genes encoding proteins either homologous or heterologous to the host cell. The signal peptide provides for extracellular expression of the truncated cellulase or derivatives thereof. The DNA signal sequence is preferably the signal sequence naturally associated with the truncated gene to be expressed, however the signal sequence from any cellobiohydrolases or endoglucanase is contemplated in the present invention.

The procedures used to ligate the DNA sequences coding for the truncated cellulases, derivatives thereof or other novel cellulases of the present invention with the promoter, and insertion into suitable vectors containing the necessary information for replication in the host cell are well known in the art.

The DNA vector or construct described above may be introduced in the host cell in accordance with known techniques such as transformation, transfection, microinjection, microporation, biolistic bombardment and the like.

In the preferred transformation technique, it must be taken into account that since the permeability of the cell wall in Trichoderma sp. is very low, uptake of the desired DNA sequence, gene or gene fragment is at best minimal. There are a number of methods to increase the permeability of the

Trichoderma sp. cell wall in the derivative strain (i.e., lacking a functional gene corresponding to the used selectable marker) prior to the transformation process.

The preferred method in the present invention to prepare Trichoderma sp. for transformation involves the preparation of protoplasts from fungal mycelium. The mycelium can be obtained from germinated vegetative spores. The mycelium is treated with an enzyme which digests the cell wall resulting in protoplasts. The protoplasts are then protected by the presence of an osmotic stabilizer in the suspending medium. These stabilizers include sorbitol, mannitol, potassium chloride, magnesium sulfate and the like. Usually the concentration of these stabilizers varies between 0.8 M to 1.2 M. It is preferable to use about a 1.2 M solution of sorbitol in the suspension medium.

Uptake of the DNA into the host Trichoderma sp. strain is dependent upon the calcium ion concentration. Generally between about 10 Mm $CaCl_2$ and 50 Mm $CaCl_2$ is used in an uptake solution. Besides the need for the calcium ion in the uptake solution, other items generally included are a buffering system such as TE buffer (10 Mm Tris, Ph 7.4; 1 Mm EDTA) or 10 Mm MOPS, Ph 6.0 buffer (morpholinepropanesulfonic acid) and polyethylene glycol (PEG). It is believed that the polyethylene glycol acts to fuse the cell membranes thus permitting the contents of the medium to be delivered into the cytoplasm of the Trichoderma sp. strain and the plasmid DNA is transferred to the nucleus. This fusion frequently leaves multiple copies of the plasmid DNA tandemly integrated into the host chromosome.

Usually a suspension containing the Trichoderma sp. protoplasts or cells that have been subjected to a permeability treatment at a density of 10^8 to 10^9/ml, preferably 2 x 10^8/ml are used in transformation. These protoplasts or cells are added to the uptake solution, along with the desired linearized selectable marker having substantially homologous flanking regions on either side of said marker to form a transformation mixture. Generally a

high concentration of PEG is added to the uptake solution. From 0.1 to 1 volume of 25% PEG 4000 can be added to the protoplast suspension. However, it is preferable to add about 0.25 volumes to the protoplast suspension. Additives such as dimethyl sulfoxide, heparin, spermidine, potassium chloride and the like may also be added to the uptake solution and aid in transformation.

Generally, the mixture is then incubated at approximately 0°C for a period between 10 to 30 minutes. Additional PEG is then added to the mixture to further enhance the uptake of the desired gene or DNA sequence. The 25% PEG 4000 is generally added in volumes of 5 to 15 times the volume of the transformation mixture; however, greater and lesser volumes may be suitable. The 25% PEG 4000 is preferably about 10 times the volume of the transformation mixture. After the PEG is added, the transformation mixture is then incubated at room temperature before the addition of a sorbitol and $CaCl_2$ solution. The protoplast suspension is then further added to molten aliquots of a growth medium. This growth medium permits the growth of transformants only. Any growth medium can be used in the present invention that is suitable to grow the desired transformants. However, if $\underline{Pyr}^+$ transformants are being selected it is preferable to use a growth medium that contains no uridine. The subsequent colonies are transferred and purified on a growth medium depleted of uridine.

At this stage, stable transformants were distinguished from unstable transformants by their faster growth rate and the formation of circular colonies with a smooth, rather than ragged outline on solid culture medium lacking uridine. Additionally, in some cases a further test of stability was made by growing the transformants on solid non-selective medium (i.e. containing uridine), harvesting spores from this culture medium and determining the percentage of these spores which will subsequently germinate and grow on selective medium lacking uridine.

In a particular embodiment of the above method, the truncated cellulases or derivatives thereof are recovered in

active form from the host cell either as a result of the appropriate post translational processing of the novel truncated cellulase or derivative thereof.

The present invention further relates to DNA gene fragments or variant DNA fragments derived from Trichoderma sp. that code for the truncated cellulase proteins or truncated cellulase protein derivatives, respectively. The DNA gene fragment or variant DNA fragment of the present invention codes for the core or binding domains of a Trichoderma sp. cellulase or derivative thereof that additionally retains the functional activity of the truncated core or binding domain, respectively. Moreover, the DNA fragment or variant thereof comprisng the sequence of the core or binding domain regions may additionally have attached thereto a linker, or hinge region DNA sequence or portion thereof wherein the encoded truncated cellulase still retains either cellulase core or binding domain activity, respectively. Furthermore, it is contemplated that additional DNA sequences that encode other proteins or enzymes of interest may be attached to the truncated DNA gene fragment or variant DNA fragment such that by following the above method of construction of vectors and expression of proteins, truncated cellulases or derivatives thereof fused to intact enzymes or proteins may be recovered. The expressed truncated cellulase fused to enzyme or protein would still retain active cellulase binding or core activity, depending on the truncated cellulase chosen to complex with the enzyme/protein.

The use of the cellulose binding domains and cellulase catalytic core domains or derivatives thereof versus using the intact cellulase enzyme may be of benefit in multiple applications. Therefore, a further aspect of the present invention is to provide methods that employ novel truncated cellulases or derivatives of truncated cellulases which provide additional benefits to the applied substrate as compared to intact cellulases. Such applications include stonewashing or biopolishing where it is contemplated that dye/colorant/pigment backstraining or redeposition can be

reduced or eliminated by employing novel truncated cellulase enzymes which have been modified so as to be devoid of a cellulose binding domain or to possess a binding domain with significantly lower affinity for cellulose, for example. In addition, it is contemplated that activity on certain substrates of interest in the textile, detergent, pulp & paper, animal feed, food, biomass industries, for example, can be significantly enhanced or diminished if the binding domain is removed or modified so as to reduce the binding affinity of the enzyme for cellulose. Also, the use of a truncated cellulase or derivative thereof described in the present invention which comprises a functional binding domain fragment, devoid of a catalytic domain or a functioning catalytic domain, may be of benefit in applications where only selected modification of the cellulosic substrate is desired. Properties which could be modified include, for example, hydration, swelling, dye diffusion and uptake, hand, friction, softness, cleaning, and/or surface or structural modification.

It is further contemplated that expression and use of some catalytic domains of cellulase enzymes would provide improved recoverability of enzyme, selectivity where lower activity on more crystalline substrate is desired or selectivity where high activity on amorphous/soluble substrate is desired.

Furthermore, catalytic domains of cellulase enzymes may be useful to enhance synergy with other cellulase components, cellulase or non-cellulase domains, and/or other enzymes or portions thereof on cellulosics cellulose containing materials in applications such as biomass conversion, cleaning, stonewashing, biopolishing of textiles, softening, pulp/paper processing, animal feed utilization, plant protection and pest control, starch processing, or production of pharmaceutical intermediates, disaccharides, or oligosaccharides.

Moreover, uses of cellulase catalytic core domains or derivatives thereof may reduce some of the detrimental properties associated with the intact enzyme on cellulosics such as pulps, cotton or other fibers, or paper. Properties

of interest include fiber/fabric strength loss, fiber/fabric weight loss, lint generation, and fibrillation damage.

It is further contemplated that cellulase catalytic core domains may exhibit less fiber roughing or reduced colorant redeposition/backstaining. Furthermore, these truncated catalytic core cellulases or derivatives thereof may offer an option for improved recovery/recycling of these novel cellulases.

Additionally, it is contemplated that the cellulase catalytic core domains or derivatives thereof in the present invention may contain selective activity advantages where hydrolysis of the soluble or more amorphous cellulosic regions of the substrate is desired but hydrolysis of the more crystalline region is not. This may be of importance in applications such as bioconversion where selective modification of the grain/fibers/plant materials is of interest.

Yet another aspect for applying the novel cellulase catalytic core domains or derivatives is in the generation of microcrystalline cellulose (MCC). Furthermore, it is contemplated that the MCC will contain less bound enzyme or that the bound enzyme may be more easibly removed.

It is further contemplated that novel covalently linked truncated cellulase domain derivatives described above may have application in controlling the access of an enzyme or modified enzyme to a substrate. This may include controlling the access of proteases to wool or other materials which contain protease substrates, or controlling the access of cellulose to cellulosics, for example.

Finally, it is contemplated that novel truncated cellulases or derivatives thereof may be applied in unique mono-, dual, or multienzyme systems. As examples this may include linking cellulase domains with each other and/or with one or more protease, cellulase, lipase, and/or amylase enzymes. The enzymes or cellulase domains may be fused with a linker region in between. This linker region may be a peptide of no functional benefit or may contain the cellulose binding

domain peptide or a peptide with high affinity for other substrates or substances, such as wool, xylan, mannan, resins, lignins, dyes, colorants, pigments, waxes, plastics, carbohydrate polymers, lipids, amino acid polymers, synthetic polymers, for example.

It is contemplated that novel cellulase domains or derivatives thereof of the present invention may provide some performance properties similar to or in excess of the intact enzyme. The novel truncated cellulases may provide these properties alone or may show synergistic benefits with cellulases or cellulase cores, other enzymes (for example, lipases, proteases, amylases, xylanases, peroxidases, reductases, esterases), other proteins or chemicals. These properties may include roughening or smoothening of the cellulosic surface, modification of the cellulosics for improved response to other enzymes such as in cleaning or pulp processing, animal feed utilization or for improved biochemical/chemical uptake by cellulosics (including plant cell walls).

It is yet further contemplated that truncated cellulase binding domains, derivatives thereof or truncated covalently linked cellulase domain derivatives in the present invention may provide enhanced or synergistic activity on cellulosics with endoglucanases and/or exocellobiohydrolases, modified cellulases or complete cellulase systems. They may also provide adhesive properties in linking cellulosic materials.

Moreover, it is contemplated that novel truncated cellulase binding domains or derivatives or the covalently linked truncated cellulase domain derivatives thereof may find application as new ligands for purification purposes, as reagents or ligands for modification of cellulosics, or other polymers, for example, linking colorants, dyes, inks, finishers, resins, chemicals, biochemicals or proteins to cellulosics. These materials can be removed at any stage, if desired, with proteases or other chemical methods. In addition, it is contemplated that the novel truncated cellulase binding domains or covalently linked truncated

cellulose domain derivatives may be used in detection and analysis of trace levels of substances, for example, the truncated domains and derivatives as well as the covalently linked truncated cellulase domain derivatives may contain proteins or chemicals which react with or bind to a substance causing it visualization e.g., dye.

Finally, it is contemplated that novel truncated binding or core domain cellulases or derivatives thereof may be complexed or fused to intact cellulases, other cellulase core or binding domains or other enzymes/proteins to improve stability, or other performance properties such as modification of pH or temperature activity profiles.

All publications and patent applications mentioned in this specification are herein incorporated by reference.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given with the understanding that they are being offered to illustrate the present invention and should not be construed in any way as limiting its scope.

EXAMPLES

Example 1.

Cloning and Expression of EG1 Core Domain Using its Own Promoter, Terminator and Signal Sequence.

Part 1. Cloning.

The complete egl1 gene used in the construction of the EG1 core domain expression plasmid, PEG1Δ3'pyr, was obtained from the plasmid PUC218::EG1. (See FIG.6.) The 3' terminator region of egl1 was ligated into PUC218 (Korman, D. et al Curr Genet 17:203-212, 1990) as a 300 bp BsmI-EcoRI fragment along with a synthetic linker designed to replace the 3' intron and cellulose binding domain with a stop codon and continue with the egl1 terminator sequences. The resultant plasmid, PEG1T, was digested with HindIII and BsmI and the vector fragment was isolated from the digest by agarose gel electrophoresis

followed by electroelution. The egl1 gene promoter sequence and core domain of egl1 were isolated from PUC218::EG1 as a 2.3kb HindIII-SstI fragment and ligated with the same synthetic linker fragment and the HindIII-BsmI digested PEG1T to form PEG1Δ3'

The net result of these operations is to replace the 3' intron and cellulose binding domain of egl1 with synthetic oligonucleotides of 53 and 55bp. These place a TAG stop codon after serine 415 and thereafter continued with the egl1 terminator up to the BsmI site.

Next, the T. longibrachiatum selectable marker, pyr4, was obtained from a previous clone p219M (Smith et al 1991), as an isolated 1.6kb EcoRI-HindIII fragment. This was incorporated into the final expression plasmid, PEG1Δ3'pyr, in a three way ligation with PUC18 plasmid digested with EcoRI and dephosphorylated using calf alkaline phosphatase and a HindIII-EcoRI fragment containing the egl1 core domain from PEG1Δ3'.

Part 2. Transformation and Expression.

A large scale DNA prep was made of PEG1Δ3'pyr and from this the EcoRI fragment containing the egl1 core domain and pyr4 gene was isolated by preparative gel electrophoresis. The isolated fragment was transformed into the uridine auxotroph version of the quad deleted strain, 1A52 pyr13 (described in U.S. Patent Application Serial Nos. 07/770,049, 08/048,728 and 08/048,881, incorporated by reference in its entirety herein), and stable transformants were identified.

To select which transformants expressed egl1 core domain the transformants were grown up in shake flasks under conditions that favored induction of the cellulase genes (Vogels + 1% lactose). After 4-5 days of growth, protein from the supernatants was concentrated and either 1) run on SDS polyacrylamide gels prior to detection of the egl1 core domain by Western analysis using EGI polyclonal antibodies or 2) the concentrated supernatants were assayed directly using RBB carboxy methyl cellulose as an endoglucanase specific

substrate and the results compared to the parental strain 1A52 as a control. Transformant candidates were identified as possibly producing a truncated EGI core domain protein. Genomic DNA and total MRNA was isolated from these strains following growth on Vogels + 1% lactose and Southern and Northern blot experiments performed using an isolated DNA fragment containing only the egl1 core domain. These experiments demonstrated that transformants could be isolated having a copy of the egl1 core domain expression cassette integrated into the genome of 1A52 and that these same transformants produced egl1 core domain MRNA.

One transformant was then grown using media suitable for cellulase production in Trichoderma well known in the art that was supplemented with lactose (Warzymoda, M. et al 1984 French Patent No. 2555603) in a 14L fermentor. The resultant broth was concentrated and the proteins contained therein were separated by SDS polyacrylamide gel electrophoresis and the Egl1 core domain protein identified by Western analysis. (See Example 3 below). It was subsequently estimated that the protein concentration of the fermentation supernatant was about 5-6 g/L of which approximately 1.7-4.4g/L was EGI core domain based on CMCase activity. This value is based on an average of several EGI core fermentations that were performed.

In a similar manner, any other cellulase domain or derivative thereof may be produced by procedures similar to those discussed above.

Example 2.

Purification of EGI and EGII catalytic cores

Part 1. EGI catalytic core

The EGI core was purified in the following manner. The concentrated (UF) broth was filtered using diatomaceous earth and ammonium sulfate was added to the broth to a final concentration of 1M (NH4)2S04. This was then loaded onto a hydrophobic column (phenyl-sepharose fast flow, Pharmacia, cat # 17-0965-02) and eluted with a salt gradient from 1M to OM

$(NH4)_2SO4$. The fractions which contained the EGI core were then pooled and exchanged into 10 mM TES pH 7.5. This solution was then loaded onto an anion exchange column (Q-sepharose fast flow, Pharmacia Cat # 17-0510-01) and eluted in a gradient from 0 to 1M NaCl in 10 mM TES pH 7.5. The most pure fractions were desalted into 10 mM TES pH 7.5 and loaded onto a MONO Q column. The EGI core elution was carried out with a gradient from 0 to 1M NaCl. The resulting fractions were greater than 85% pure. The most pure fraction was sequence verified to be the EGI core.

Part 2. EGII catalytic core

It is contemplated that the purification of the EGII catalytic core is similar to that of EGII cellulase because of its similar biochemical properties. The theoretical pI of the EGII core is less than a half a pH unit lower than that of EGII. Also, EGII core is approximately 80% of the molecular weight of EGII. Therefore, the following purification protocol is based on the purification of EGII. The method may involve filtering the UF concentrated broth through diatomaceous earth and adding (NH4)2S04 to bring the solution to 1M (NH4)2S04. This solution may then be loaded onto a hydrophobic column (phenyl-sepharose fast flow, Pharmacia, cat #17-0965-02) and the EGII may be step eluted with 0.15 M (NH4)2S04. The fractions containing the EGII core may then be buffer exchanged into citrate-phosphate pH 7, 0.18 mOhm. This material may then be loaded onto a anion exchange column (Q-sepharose fast flow, Pharmacia, cat. #17-0510-01) equilibrated in the above citrate-phosphate buffer. It is expected that EGII core will not bind to the column and thus be collected in the flow through.

Example 3.

Cloning and Expression of CBHII Core Domain Using the CBHI Promoter, Terminator and Signal Sequence from CBHII.

Part 1. Construction of the T.longibrachiatum general-purpose expression plasmid-PTEX.

The plasmid, PTEX was constructed following the methods of Sambrook et al. (1989), supra, and is illustrated in FIG. 7. This plasmid has been designed as a multi-purpose expression vector for use in the filamentous fungus Trichoderma longibrachiatum. The expression cassette has several unique features that make it useful for this function. Transcription is regulated using the strong CBH I gene promoter and terminator sequences for T. longibrachiatum. Between the CBHI promoter and terminator there are unique PmeI and SstI restriction sites that are used to insert the gene to be expressed. The T. longibrachiatum pyr4 selectable marker gene has been inserted into the CBHI terminator and the whole expression cassette (CBHI promoter-insertion sites-CBHI terminator-pyr4 gene-CBHI terminator) can be excised utilizing the unique NotI restriction site or the unique NotI and NheI restriction sites.

This vector is based on the bacterial vector, pSL1180 (Pharmacia Inc., Piscataway, New Jersey), which is a PUC-type vector with an extended multiple cloning site. One skilled in the art would be able to construct this vector based on the flow diagram illustrated in FIG 7. (See also U.S. patent application 07/954,113 for the construction of PTEX expression plasmid.)

It would be possible to construct plasmids similar to PTEX-truncated cellulases or derivatives thereof described in the present invention containing any other piece of DNA sequence replacing the truncated cellulase gene.

Part 2. Cloning.

The complete cbh2 gene used in the construction of the CBHII core domain expression plasmid, PTEX CBHII core, was obtained from the plasmid PUC219::CBHII (Korman, D. et al, 1990, Curr Genet 17:203-212). The cellulose binding domain, positioned at the 5′ end of the cbh2 gene, is conveniently located between an XbaI and SnaBI restriction sites. In order to utilize the XbaI site an additional XbaI site in the polylinker was destroyed. PUC219::CBHII was partially digested with XbaI such that the majority of the product was linear. The XbaI overhangs were filled in using T4 DNA polymerase and ligated together under conditions favoring self ligation of the plasmid. This has the effect of destroying the blunted site which, in 50% of the plasmids, was the XbaI site in the polylinker. Such a plasmid was identified and digested with XbaI and SnaBI to release the cellulose binding domain. The vector-CBHII core domain was isolated and ligated with the following synthetic oligonucleotides designed to join the XbaI site with the SnaBI site at the signal peptidase cleavage site and papain cleavage point in the linker domain.

XbaI SnaBI

5′ CTA GAG CGG TCG GGA ACC GCT AC 3′ (Seq ID No: 44)

3′ TC CTC GCC AGC CCT TGG CGA TG 5′

Leu Glu Glu Arg Ser Gly Thr Ala Thr (Seq ID No: 45)

The resultant plasmid, pUCΔCBD CBHII, was digested with NheI and the ends blunted by incubation with T4 DNA polymerase and dNTPs. After which the linear blunted plasmid DNA was digested with BglII and the Nhe (blunt) BglII fragment containing the CBHII signal sequence and core domain was isolated.

The final expression plasmid was engineered by digesting the general purpose expression plasmid, pTEX (disclosed in 07/954,113, incorporated in its entirety by references, and described in Part 3 below), with SstII and PmeI and ligating the CBHII NheI (blunt)-BglII fragment downstream of the cbh1

promoter using a synthetic oligonucleotide having the sequence CGCTAG to fill in the BglII overhang with the SstII overhang.

The pTEX-CBHI core expression plasmid was prepared in a similar manner as pTEX-CBHII core described in the above example. Its construction is exemplified in Figure 8.

Part 3. Transformation and Expression.

A large scale DNA prep was made of pTEX CBHIIcore and from this the NotI fragment containing the CBHII core domain under the control of the cbh1 transcriptional elements and pyr4 gene was isolated by preparative gel electrophoresis. The isolated fragment was transformed into the uridine auxotroph version of the quad deleted strain, 1A52 pyr13, and stable transformants were identified.

To select which transformants expressed cbh2 core domain genomic DNA was isolated from strains following growth on Vogels + 1% glucose and Southern blot experiments performed using an isolated DNA fragment containing only the cbh2 core domain. Transformants were isolated having a copy of the cbh2 core domain expression cassette integrated into the genome of 1A52. Total mRNA was isolated from the two strains following growth for 1 day on Vogels + 1% lactose. The mRNA was subjected to Northern analysis using the cbh2 coding region as a probe. Transformants expressing cbh2 core domain mRNA were identified.

Two transformants were grown under the same conditions as previously described in Example 1 in 14L fermentors. The resultant broth was concentrated and the proteins contained therein were separated by SDS polyacrylamide gel electrophoresis and the CBHII core domain protein identified by Western analysis. One transformant, #15, produced a protein of the correct size and reactivity to CBHII polyclonal antibodies.

It was subsequently estimated that the protein concentration of the fermentation supernatant after purification was 10g/L of which 30-50% was CBHII core domain (See Example 4).

One may obtain any other novel truncated cellulase core domain protein or derivative thereof by employing the methods described above.

Example 4.

Purification of CBHI and CBHII catalytic cores

Part 1. CBHI catalytic core.

The CBHI core was purified from broth obtained from T. longibrachiatum harboring pTEX-CBHI core expression vector in the following manner. The CBHI core ultrafiltered (UF) broth was filtered using diatomaceous earth and diluted in 10 mM TES pH 6.8 to a conductivity of 1.5 mOhm. The diluted CBHI core was then loaded onto an anion exchange column (Q-Sepharose fast flow, Pharmacia cat # 17-0510-01) equilibrated in 10 mM TES pH 6.8 The CBHI core was separated from the majority of the other proteins in the broth using a gradient elution in 10 mM TES pH 6.8 from 0 to 1M NaCl. The fractions containing the CBHI core were then concentrated on an Amicon stirred cell concentrator with a PM 10 membrane (diaflo ultra filtration membranes, Amicon Cat # 13132MEM 5468A). This step concentrated the core as well as separated it from lower molecular weight proteins. The resulting fractions were greater than 85% pure CBHI core. The purest fraction was sequence verified to be the CBHI core.

Part 2. CBHII catalytic core.

It is predicted that CBHII catalytic core will purify in a manner similar to that of CBHII cellulase because of its similar biochemical properties. The theoretical pI of the CBHII core is less than half a pH unit lower than that of CBHII. Additionally, CBHII catalytic core is approximately 80% of the molecular weight of CBHII. Therefore, the following proposed purification protocol is based on the purification method used for CBHII. The diatomaceous earth treated, ultra filtered (UF) CBHII core broth is diluted into 10 mM TES pH 6.8 to a conductivity of <0.7 mOhm. The diluted

CBHII core is then loaded onto an anion exchange column (Q-Sepharose fast flow, Pharmacia, cat # 17 0510-01) equilibrated in 10 mM TES pH 6.8. A salt gradient from 0 to 1M NaCl in 10 mM TES pH 6.8 is used to elute the CBHII core off the column. The fractions which contain the CBHII core is then buffer exchanged into 2mM sodium succinate buffer and loaded onto a cation exchange column (SP-sephadex C-50). The CBHII core is next eluted from the column with a salt gradient from 0 to 100mM NaCl.

Example 5.

Cloning and Expression of CBHII Cellulose Binding Domain Using the CBHI Promoter.

Part 1. Cloning.

The complete cbh2 gene used in the construction of the CBHII core domain expression plasmid, pTEX CBHIIcore, was obtained from the plasmid pUC219::CBHII. The cellulose binding domain, positioned at the 5′ end of the cbh2 gene, was obtained by digestion of PUC219::CBHII with BglII and NsiI and isolating the 450bp BglII-NsiI restriction fragment. The final expression plasmid, PTEX CBHII CBD was engineered by digesting the general purpose expression plasmid, PTEX (described in 07/954,113 and incorporated herein by reference in its entirety), with SstII and PmeI and ligating the CBHII CBD BglII-NsiI fragment downstream of the cbh1 promoter using a synthetic oligonucleotide having the sequence 3′ CGCTAG 5′ to fill in the BglII overhang with the SstII overhang and the following synthetic linker to link the NsiI site with the blunt PmeI site of pTEX. (See FIG 9).

```
      5′ TAT TAC TAA 3′
3′ ACGT ATA ATG ATT  5′
   NsiI          *** *** Stop codons
```

When the final expression plasmid, pTEX CBHII CBD, was sequenced across the linker junctions it was discovered that

the sticky NsiI site had ligated directly to the blunt PmeI site in pTEX. This means that the reading frame of the CBHII CBD continues on through the PmeI linker and into the cbh1 terminator for a further 12 amino acids as follows;

5' AAA CCC CGG GTG ATT TAT TTT TTT TGT ATC TAC TTC TGA 3'
3'TTT GGG GCC CAC TAA ATA AAA AAA ACA TAG ATG AAG ACT 5'
(Seq ID No: 46)

Lys Pro Arg Val Ile Tyr Phe Phe Cys Ile Tyr Phe ***
(Seq ID No: 47)

However, the addition of these additional amino acids is not thought to significantly change the properties of the cellulose binding domain.

In a similar fashion, it is contemplated that any one of the other known binding domains may be substituted in the above pTEX construct to provide expression of the substituted binding domains by following the general format disclosed above.

Part 2. Transformation and Expression.

A large scale DNA prep was made of pTEX CBHII CBD and from this the NotI fragment containing the CBHII core domain under the control of the cbh1 transcriptional elements and pyr4 gene was isolated by preparative gel electrophoresis. The isolated fragment was transformed into the uridine auxotroph version of the quad deleted strain, 1A52 pyr13, and stable transformants were identified.

To select which transformants expressed cbh2 cellulose binding domain, genomic DNA was isolated from all stably transformant strains following growth on Vogels + 1% glucose and Southern blot experiments performed using an isolated DNA fragment containing the cbh1 gene to identify the transformants containing the CBHII CBD PTEX expression vector. Total mRNA was isolated from the transformed strains following growth for 1 day on Vogels + 1% lactose. The MRNA was subjected to Northern analysis using the cbh2 coding region as a probe. Most of the transformants expressed cbh2

CBD MRNA at high levels. One transformant was selected and grown under conditions previously described in a 14L fermentor. The resultant broth was concentrated and the proteins contained therein were separated by SDS polyacrylamide gel electrophoresis and the CBHII CBD protein subjected to Western analysis. A protein of the expected size was identified by reactivity to CBHII CBD polyclonal antibodies raised against the synthetic CBHII CBD peptide having the sequence;

NH2 C-G-G-Q-N-V-S-G-P-T-C-C-A-S-G-S-T-C-COOH

(Seq ID No: 48)

Example 6

Purification of Cellulose Binding Domains

The binding domain can ben purified by methods similar to those reported in the literature (Ong, E., et al 1989 Bio/Technology 7: 604-607). In the case of affinity chromatography, the filtered binding domain broth can be contacted with a cellulosic substance, such as avicel or pulp/paper. The cellulosic solids may be separated by centrifugation or filtration. Alternatively, the filtered broth may be passed over a cellulosic-type column. The bound binding domains may then be eluted by treatment with distilled water, guanidinium HCl/other denaturants, surfactants, or other appropriate elution chemicals. Use of temperature modification may also be an option. Affinity chromatography using antibodies generated against the CBD or CBD derivative may also be employed. A particular purification procedure may require several fractionation steps depending upon the sample matrix and upon the chemical properties of the binding domains and modified domains of the present invention. In some cases the modified domains may contain additional charged functional groups which may allow for the use of other methods such as ionic exchange.

While the invention has been described in terms of various preferred embodiments, the skilled artisan will

appreciate that various modifications, substitutions, omissions, and changes may be made without departing from the scope and spirit thereof. Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims, including equivalents thereof.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Fowler, Timothy
Ward, Michael
Clarkson, Kathleen
Collier, Katherine
Larenas, Edmund

(ii) TITLE OF INVENTION: Novel Cellulase Enzymes and Systems For Their Expression

(iii) NUMBER OF SEQUENCES: 48

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genencor International
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(C) CITY: South San Francisco
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(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/169,948
(B) FILING DATE: DEC 17 1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Horn, Margaret A.
(B) REGISTRATION NUMBER: 33,401
(C) REFERENCE/DOCKET NUMBER: GC226

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 742-7536
(B) TELEFAX: (415)742-7217

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..93

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGC CAG TGC GGC GGT ATT GGC TAC AGC GGC CCC ACG GTC TGC GCC AGC        48
Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser
  1               5                  10                  15

GGC ACA ACT TGC CAG GTC CTG AAC CCT TAC TAC TCT CAG TGC CTG            93
Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys Leu
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser
  1               5                  10                  15

Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys Leu
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..20, 70..166)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CAA GCT TGC TCA AGC GTC  TG  GTAATTATGT GAACCCTCTC AAGAGACCCA          50
Gln Ala Cys Ser Ser Val  Trp
  1               5

AATACTGAGA TATGTCAAG G GGC CAA TGT GGT GGC CAG AAT TGG TCG GGT        100
                      Gly Gln Cys Gly Gly Gln Asn Trp Ser Gly
                                  10                  15

CCG ACT TGC TGT GCT TCC GGA AGC ACA TGC GTC TAC TCC AAC GAC TAT       148
Pro Thr Cys Cys Ala Ser Gly Ser Thr Cys Val Tyr Ser Asn Asp Tyr
      20                  25                  30

TAC TCC CAG TGT CTT CCC                                              166
Tyr Ser Gln Cys Leu Pro
     35
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Gln Ala Cys Ser Ser Val Trp Gly Gln Cys Gly Gly Gln Asn Trp Ser
 1               5                  10                  15

Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr Cys Val Tyr Ser Asn Asp
            20                  25                  30

Tyr Tyr Ser Gln Cys Leu Pro
        35
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 156 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..82, 140..156)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CAC TGG GGG CAG TGC GGT GGC ATT GGG TAC AGC GGG TGC AAG ACG TGC      48
His Trp Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Cys Lys Thr Cys
 1               5                  10                  15

ACG TCG GGC ACT ACG TGC CAG TAT AGC AAC GAC  T GTTCGTATCC          92
Thr Ser Gly Thr Thr Cys Gln Tyr Ser Asn Asp
            20                  25

CCATGCCTGA CGGGAGTGAT TTTGAGATGC TAACCGCTAA AATACAG  AC TAC TCG    147
                                                 Tyr Tyr Ser
                                                     30

CAA TGC CTT                                                       156
Gln Cys Leu
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
His Trp Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Cys Lys Thr Cys
 1               5                  10                  15

Thr Ser Gly Thr Thr Cys Gln Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys
            20                  25                  30

Leu
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CAG CAG ACT GTC TGG GGC CAG TGT GGA GGT ATT GGT TGG AGC GGA CCT      48
Gln Gln Thr Val Trp Gly Gln Cys Gly Gly Ile Gly Trp Ser Gly Pro
 1               5                  10                  15

ACG AAT TGT GCT CCT GGC TCA GCT TGT TCG ACC CTC AAT CCT TAT TAT      96
Thr Asn Cys Ala Pro Gly Ser Ala Cys Ser Thr Leu Asn Pro Tyr Tyr
            20                  25                  30

GCG CAA TGT ATT                                                    108
Ala Gln Cys Ile
        35
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Gln Gln Thr Val Trp Gly Gln Cys Gly Gly Ile Gly Trp Ser Gly Pro
 1               5                  10                  15

Thr Asn Cys Ala Pro Gly Ser Ala Cys Ser Thr Leu Asn Pro Tyr Tyr
            20                  25                  30

Ala Gln Cys Ile
        35
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1453 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..410, 478..1174, 1238..1453)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CAG TCG GCC TGC ACT CTC CAA TCG GAG ACT CAC CCG CCT CTG ACA TGG      48
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
 1               5                  10                  15

CAG AAA TGC TCG TCT GGT GGC ACT TGC ACT CAA CAG ACA GGC TCC GTG      96
Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30

GTC ATC GAC GCC AAC TGG CGC TGG ACT CAC GCT ACG AAC AGC AGC ACG     144
Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
        35                  40                  45

AAC TGC TAC GAT GGC AAC ACT TGG AGC TCG ACC CTA TGT CCT GAC AAC     192
Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
    50                  55                  60

GAG ACC TGC GCG AAG AAC TGC TGT CTG GAC GGT GCC GCC TAC GCG TCC     240
Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65                  70                  75                  80

ACG TAC GGA GTT ACC ACG AGC GGT AAC AGC CTC TCC ATT GGC TTT GTC     288
Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
                85                  90                  95

ACC CAG TCT GCG CAG AAG AAC GTT GGC GCT CGC CTT TAC CTT ATG GCG     336
Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
            100                 105                 110

AGC GAC ACG ACC TAC CAG GAA TTC ACC CTG CTT GGC AAC GAG TTC TCT     384
Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
        115                 120                 125

TTC GAT GTT GAT GTT TCG CAG CTG  CC  GTAAGTGACT TACCATGAAC          430
Phe Asp Val Asp Val Ser Gln Leu  Pro
    130                 135

CCCTGACGTA TCTTCTTGTG GGCTCCCAGC TGACTGGCCA ATTTAAG G TGC GGC      484
                                                    Cys Gly
```

```
TTG AAC GGA GCT CTC TAC TTC GTG TCC ATG GAC GCG GAT GGT GGC GTG        532
Leu Asn Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val
140                 145                 150                 155

AGC AAG TAT CCC ACC AAC ACC GCT GGC GCC AAG TAC GGC ACG GGG TAC        580
Ser Lys Tyr Pro Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr
                160                 165                 170

TGT GAC AGC CAG TGT CCC CGC GAT CTG AAG TTC ATC AAT GGC CAG GCC        628
Cys Asp Ser Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala
            175                 180                 185

AAC GTT GAG GGC TGG GAG CCG TCA TCC AAC AAC GCA AAC ACG GGC ATT        676
Asn Val Glu Gly Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile
        190                 195                 200

GGA GGA CAC GGA AGC TGC TGC TCT GAG ATG GAT ATC TGG GAG GCC AAC        724
Gly Gly His Gly Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn
    205                 210                 215

TCC ATC TCC GAG GCT CTT ACC CCC CAC CCT TGC ACG ACT GTC GGC CAG        772
Ser Ile Ser Glu Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln
220                 225                 230                 235

GAG ATC TGC GAG GGT GAT GGG TGC GGC GGA ACT TAC TCC GAT AAC AGA        820
Glu Ile Cys Glu Gly Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg
                240                 245                 250

TAT GGC GGC ACT TGC GAT CCC GAT GGC TGC GAC TGG AAC CCA TAC CGC        868
Tyr Gly Gly Thr Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg
            255                 260                 265

CTG GGC AAC ACC AGC TTC TAC GGC CCT GGC TCA AGC TTT ACC CTC GAT        916
Leu Gly Asn Thr Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp
        270                 275                 280

ACC ACC AAG AAA TTG ACC GTT GTC ACC CAG TTC GAG ACG TCG GGT GCC        964
Thr Thr Lys Lys Leu Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala
    285                 290                 295

ATC AAC CGA TAC TAT GTC CAG AAT GGC GTC ACT TTC CAG CAG CCC AAC       1012
Ile Asn Arg Tyr Tyr Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn
300                 305                 310                 315

GCC GAG CTT GGT AGT TAC TCT GGC AAC GAG CTC AAC GAT GAT TAC TGC       1060
Ala Glu Leu Gly Ser Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys
                320                 325                 330

ACA GCT GAG GAG GCA GAA TTC GGC GGA TCC TCT TTC TCA GAC AAG GGC       1108
Thr Ala Glu Glu Ala Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly
            335                 340                 345

GGC CTG ACT CAG TTC AAG AAG GCT ACC TCT GGC GGC ATG GTT CTG GTC       1156
Gly Leu Thr Gln Phe Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val
        350                 355                 360

ATG AGT CTG TGG GAT GAT GTGAGTTTGA TGGACAAACA TGCGCGTTGA              1204
Met Ser Leu Trp Asp Asp
    365

CAAAGAGTCA AGCAGCTGAC TGAGATGTTA CAG TAC TAC GCC AAC ATG CTG TGG       1258
                                  Tyr Tyr Ala Asn Met Leu Trp
                                  370                 375

CTG GAC TCC ACC TAC CCG ACA AAC GAG ACC TCC TCC ACA CCC GGT GCC       1306
```

```
Leu Asp Ser Thr Tyr Pro Thr Asn Glu Thr Ser Ser Thr Pro Gly Ala
            380                 385                 390

GTG CGC GGA AGC TGC TCC ACC AGC TCC GGT GTC CCT GCT CAG GTC GAA      1354
Val Arg Gly Ser Cys Ser Thr Ser Ser Gly Val Pro Ala Gln Val Glu
        395                 400                 405

TCT CAG TCT CCC AAC GCC AAG GTC ACC TTC TCC AAC ATC AAG TTC GGA      1402
Ser Gln Ser Pro Asn Ala Lys Val Thr Phe Ser Asn Ile Lys Phe Gly
    410                 415                 420

CCC ATT GGC AGC ACC GGC AAC CCT AGC GGC GGC AAC CCT CCC GGC GGA      1450
Pro Ile Gly Ser Thr Gly Asn Pro Ser Gly Gly Asn Pro Pro Gly Gly
425                 430                 435                 440

AAC                                                                  1453
Asn
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr Trp
  1               5                  10                  15

Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser Val
            20                  25                  30

Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser Thr
        35                  40                  45

Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp Asn
    50                  55                  60

Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
65                  70                  75                  80

Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val
                85                  90                  95

Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala
            100                 105                 110
```

Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser
115 120 125

Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala Leu
130 135 140

Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro Thr
145 150 155 160

Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln Cys
165 170 175

Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly Trp
180 185 190

Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
195 200 205

Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
210 215 220

Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly
225 230 235 240

Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys
245 250 255

Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser
260 265 270

Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu
275 280 285

Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr
290 295 300

Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser
305 310 315 320

Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala
325 330 335

Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe
340 345 350

Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp Asp
355 360 365

Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn
370 375 380

Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser
385 390 395 400

Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val
405 410 415

Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro
420 425 430

Ser Gly Gly Asn Pro Pro Gly Gly Asn
435 440

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1241 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..161, 218..465, 556..1241)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TCG GGA ACC GCT ACG TAT TCA GGC AAC CCT TTT GTT GGG GTC ACT CCT        48
Ser Gly Thr Ala Thr Tyr Ser Gly Asn Pro Phe Val Gly Val Thr Pro
 1               5                  10                  15

TGG GCC AAT GCA TAT TAC GCC TCT GAA GTT AGC AGC CTC GCT ATT CCT        96
Trp Ala Asn Ala Tyr Tyr Ala Ser Glu Val Ser Ser Leu Ala Ile Pro
            20                  25                  30

AGC TTG ACT GGA GCC ATG GCC ACT GCT GCA GCA GCT GTC GCA AAG GTT       144
Ser Leu Thr Gly Ala Met Ala Thr Ala Ala Ala Ala Val Ala Lys Val
        35                  40                  45

CCC TCT TTT ATG TGG  CT  GTAGGTCCTC CCGGAACCAA GGCAATCTGT             191
Pro Ser Phe Met Trp  Leu
    50

TACTGAAGGC TCATCATTCA CTGCAG A GAT ACT CTT GAC AAG ACC CCT CTC        242
                              Asp Thr Leu Asp Lys Thr Pro Leu
                               55                  60

ATG GAG CAA ACC TTG GCC GAC ATC CGC ACC GCC AAC AAG AAT GGC GGT       290
Met Glu Gln Thr Leu Ala Asp Ile Arg Thr Ala Asn Lys Asn Gly Gly
        65                  70                  75

AAC TAT GCC GGA CAG TTT GTG GTG ATA GAC TTG CCG GAT CGC GAT TGC       338
Asn Tyr Ala Gly Gln Phe Val Val Ile Asp Leu Pro Asp Arg Asp Cys
    80                  85                  90

GCT GCC CTT GCC TCG AAT GGC GAA TAC TCT ATT GCC GAT GGT GGC GTC       386
Ala Ala Leu Ala Ser Asn Gly Glu Tyr Ser Ile Ala Asp Gly Gly Val
95                  100                 105                 110

GCC AAA TAT AAG AAC TAT ATC GAC ACC ATT CGT CAA ATT GTC GTG GAA       434
Ala Lys Tyr Lys Asn Tyr Ile Asp Thr Ile Arg Gln Ile Val Val Glu
                115                 120                 125

TAT TCC GAT ATC CGG ACC CTC CTG GTT ATT  G GTATGAGTTT AAACACCTGC      485
Tyr Ser Asp Ile Arg Thr Leu Leu Val Ile
            130                 135

CTCCCCCCCC CCTTCCCTTC CTTTCCCGCC GGCATCTTGT CGTTGTGCTA ACTATTGTTC     545
```

```
CCTCTTCCAG  AG CCT GAC TCT CTT GCC AAC CTG GTG ACC AAC CTC GGT        593
            Glu Pro Asp Ser Leu Ala Asn Leu Val Thr Asn Leu Gly
                        140                 145

ACT CCA AAG TGT GCC AAT GCT CAG TCA GCC TAC CTT GAG TGC ATC AAC        641
Thr Pro Lys Cys Ala Asn Ala Gln Ser Ala Tyr Leu Glu Cys Ile Asn
150                 155                 160                 165

TAC GCC GTC ACA CAG CTG AAC CTT CCA AAT GTT GCG ATG TAT TTG GAC        689
Tyr Ala Val Thr Gln Leu Asn Leu Pro Asn Val Ala Met Tyr Leu Asp
                170                 175                 180

GCT GGC CAT GCA GGA TGG CTT GGC TGG CCG GCA AAC CAA GAC CCG GCC        737
Ala Gly His Ala Gly Trp Leu Gly Trp Pro Ala Asn Gln Asp Pro Ala
            185                 190                 195

GCT CAG CTA TTT GCA AAT GTT TAC AAG AAT GCA TCG TCT CCG AGA GCT        785
Ala Gln Leu Phe Ala Asn Val Tyr Lys Asn Ala Ser Ser Pro Arg Ala
        200                 205                 210

CTT CGC GGA TTG GCA ACC AAT GTC GCC AAC TAC AAC GGG TGG AAC ATT        833
Leu Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr Asn Gly Trp Asn Ile
    215                 220                 225

ACC AGC CCC CCA TCG TAC ACG CAA GGC AAC GCT GTC TAC AAC GAG AAG        881
Thr Ser Pro Pro Ser Tyr Thr Gln Gly Asn Ala Val Tyr Asn Glu Lys
230                 235                 240                 245

CTG TAC ATC CAC GCT ATT GGA CCT CTT CTT GCC AAT CAC GGC TGG TCC        929
Leu Tyr Ile His Ala Ile Gly Pro Leu Leu Ala Asn His Gly Trp Ser
                250                 255                 260

AAC GCC TTC TTC ATC ACT GAT CAA GGT CGA TCG GGA AAG CAG CCT ACC        977
Asn Ala Phe Phe Ile Thr Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
            265                 270                 275

GGA CAG CAA CAG TGG GGA GAC TGG TGC AAT GTG ATC GGC ACC GGA TTT       1025
Gly Gln Gln Gln Trp Gly Asp Trp Cys Asn Val Ile Gly Thr Gly Phe
        280                 285                 290

GGT ATT CGC CCA TCC GCA AAC ACT GGG GAC TCG TTG CTG GAT TCG TTT       1073
Gly Ile Arg Pro Ser Ala Asn Thr Gly Asp Ser Leu Leu Asp Ser Phe
    295                 300                 305

GTC TGG GTC AAG CCA GGC GGC GAG TGT GAC GGC ACC AGC GAC AGC AGT       1121
Val Trp Val Lys Pro Gly Gly Glu Cys Asp Gly Thr Ser Asp Ser Ser
310                 315                 320                 325

GCG CCA CGA TTT GAC TCC CAC TGT GCG CTC CCA GAT GCC TTG CAA CCG       1169
Ala Pro Arg Phe Asp Ser His Cys Ala Leu Pro Asp Ala Leu Gln Pro
                330                 335                 340

GCG CCT CAA GCT GGT GCT TGG TTC CAA GCC TAC TTT GTG CAG CTT CTC       1217
Ala Pro Gln Ala Gly Ala Trp Phe Gln Ala Tyr Phe Val Gln Leu Leu
            345                 350                 355

ACA AAC GCA AAC CCA TCG TTC CTG                                      1241
Thr Asn Ala Asn Pro Ser Phe Leu
        360                 365
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Ser Gly Thr Ala Thr Tyr Ser Gly Asn Pro Phe Val Gly Val Thr Pro
 1               5                  10                  15

Trp Ala Asn Ala Tyr Tyr Ala Ser Glu Val Ser Ser Leu Ala Ile Pro
            20                  25                  30

Ser Leu Thr Gly Ala Met Ala Thr Ala Ala Ala Ala Val Ala Lys Val
        35                  40                  45

Pro Ser Phe Met Trp Leu Asp Thr Leu Asp Lys Thr Pro Leu Met Glu
    50                  55                  60

Gln Thr Leu Ala Asp Ile Arg Thr Ala Asn Lys Asn Gly Gly Asn Tyr
65                  70                  75                  80

Ala Gly Gln Phe Val Val Ile Asp Leu Pro Asp Arg Asp Cys Ala Ala
                85                  90                  95

Leu Ala Ser Asn Gly Glu Tyr Ser Ile Ala Asp Gly Gly Val Ala Lys
            100                 105                 110

Tyr Lys Asn Tyr Ile Asp Thr Ile Arg Gln Ile Val Val Glu Tyr Ser
        115                 120                 125

Asp Ile Arg Thr Leu Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Leu
    130                 135                 140

Val Thr Asn Leu Gly Thr Pro Lys Cys Ala Asn Ala Gln Ser Ala Tyr
145                 150                 155                 160

Leu Glu Cys Ile Asn Tyr Ala Val Thr Gln Leu Asn Leu Pro Asn Val
                165                 170                 175

Ala Met Tyr Leu Asp Ala Gly His Ala Gly Trp Leu Gly Trp Pro Ala
            180                 185                 190

Asn Gln Asp Pro Ala Ala Gln Leu Phe Ala Asn Val Tyr Lys Asn Ala
        195                 200                 205

Ser Ser Pro Arg Ala Leu Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr
    210                 215                 220

Asn Gly Trp Asn Ile Thr Ser Pro Pro Ser Tyr Thr Gln Gly Asn Ala
225                 230                 235                 240

Val Tyr Asn Glu Lys Leu Tyr Ile His Ala Ile Gly Pro Leu Leu Ala
                245                 250                 255

Asn His Gly Trp Ser Asn Ala Phe Phe Ile Thr Asp Gln Gly Arg Ser
            260                 265                 270

Gly Lys Gln Pro Thr Gly Gln Gln Gln Trp Gly Asp Trp Cys Asn Val
        275                 280                 285

Ile Gly Thr Gly Phe Gly Ile Arg Pro Ser Ala Asn Thr Gly Asp Ser
    290                 295                 300
```

```
Leu Leu Asp Ser Phe Val Trp Val Lys Pro Gly Gly Glu Cys Asp Gly
305                 310                 315                 320

Thr Ser Asp Ser Ser Ala Pro Arg Phe Asp Ser His Cys Ala Leu Pro
                325                 330                 335

Asp Ala Leu Gln Pro Ala Pro Gln Ala Gly Ala Trp Phe Gln Ala Tyr
            340                 345                 350

Phe Val Gln Leu Leu Thr Asn Ala Asn Pro Ser Phe Leu
        355                 360                 365
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1201 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..704, 775..1201)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CAG CAA CCG GGT ACC AGC ACC CCC GAG GTC CAT CCC AAG TTG ACA ACC        48
Gln Gln Pro Gly Thr Ser Thr Pro Glu Val His Pro Lys Leu Thr Thr
  1               5                  10                  15

TAC AAG TGT ACA AAG TCC GGG GGG TGC GTG GCC CAG GAC ACC TCG GTG        96
Tyr Lys Cys Thr Lys Ser Gly Gly Cys Val Ala Gln Asp Thr Ser Val
            20                  25                  30

GTC CTT GAC TGG AAC TAC CGC TGG ATG CAC GAC GCA AAC TAC AAC TCG       144
Val Leu Asp Trp Asn Tyr Arg Trp Met His Asp Ala Asn Tyr Asn Ser
        35                  40                  45

TGC ACC GTC AAC GGC GGC GTC AAC ACC ACG CTC TGC CCT GAC GAG GCG       192
Cys Thr Val Asn Gly Gly Val Asn Thr Thr Leu Cys Pro Asp Glu Ala
    50                  55                  60

ACC TGT GGC AAG AAC TGC TTC ATC GAG GGC GTC GAC TAC GCC GCC TCG       240
Thr Cys Gly Lys Asn Cys Phe Ile Glu Gly Val Asp Tyr Ala Ala Ser
65                  70                  75                  80

GGC GTC ACG ACC TCG GGC AGC AGC CTC ACC ATG AAC CAG TAC ATG CCC       288
Gly Val Thr Thr Ser Gly Ser Ser Leu Thr Met Asn Gln Tyr Met Pro
                85                  90                  95
```

```
AGC AGC TCT GGC GGC TAC AGC AGC GTC TCT CCT CGG CTG TAT CTC CTG      336
Ser Ser Ser Gly Gly Tyr Ser Ser Val Ser Pro Arg Leu Tyr Leu Leu
            100                 105                 110

GAC TCT GAC GGT GAG TAC GTG ATG CTG AAG CTC AAC GGC CAG GAG CTG      384
Asp Ser Asp Gly Glu Tyr Val Met Leu Lys Leu Asn Gly Gln Glu Leu
        115                 120                 125

AGC TTC GAC GTC GAC CTC TCT GCT CTG CCG TGT GGA GAG AAC GGC TCG      432
Ser Phe Asp Val Asp Leu Ser Ala Leu Pro Cys Gly Glu Asn Gly Ser
    130                 135                 140

CTC TAC CTG TCT CAG ATG GAC GAG AAC GGG GGC GCC AAC CAG TAT AAC      480
Leu Tyr Leu Ser Gln Met Asp Glu Asn Gly Gly Ala Asn Gln Tyr Asn
145                 150                 155                 160

ACG GCC GGT GCC AAC TAC GGG AGC GGC TAC TGC GAT GCT CAG TGC CCC      528
Thr Ala Gly Ala Asn Tyr Gly Ser Gly Tyr Cys Asp Ala Gln Cys Pro
                165                 170                 175

GTC CAG ACA TGG AGG AAC GGC ACC CTC AAC ACT AGC CAC CAG GGC TTC      576
Val Gln Thr Trp Arg Asn Gly Thr Leu Asn Thr Ser His Gln Gly Phe
            180                 185                 190

TGC TGC AAC GAG ATG GAT ATC CTG GAG GGC AAC TCG AGG GCG AAT GCC      624
Cys Cys Asn Glu Met Asp Ile Leu Glu Gly Asn Ser Arg Ala Asn Ala
        195                 200                 205

TTG ACC CCT CAC TCT TGC ACG GCC ACG GCC TGC GAC TCT GCC GGT TGC      672
Leu Thr Pro His Ser Cys Thr Ala Thr Ala Cys Asp Ser Ala Gly Cys
    210                 215                 220

GGC TTC AAC CCC TAT GGC AGC GGC TAC AAA  AG  GTGAGCCTGA              714
Gly Phe Asn Pro Tyr Gly Ser Gly Tyr Lys  Ser
225                 230                 235

TGCCACTACT ACCCCTTTCC TGGCGCTCTC GCGGTTTTCC ATGCTGACAT GGTTTTCCAG    774

C TAC TAC GGC CCC GGA GAT ACC GTT GAC ACC TCC AAG ACC TTC ACC        820
  Tyr Tyr Gly Pro Gly Asp Thr Val Asp Thr Ser Lys Thr Phe Thr
                240                 245                 250

ATC ATC ACC CAG TTC AAC ACG GAC AAC GGC TCG CCC TCG GGC AAC CTT      868
Ile Ile Thr Gln Phe Asn Thr Asp Asn Gly Ser Pro Ser Gly Asn Leu
                255                 260                 265

GTG AGC ATC ACC CGC AAG TAC CAG CAA AAC GGC GTC GAC ATC CCC AGC      916
Val Ser Ile Thr Arg Lys Tyr Gln Gln Asn Gly Val Asp Ile Pro Ser
            270                 275                 280

GCC CAG CCC GGC GGC GAC ACC ATC TCG TCC TGC CCG TCC GCC TCA GCC      964
Ala Gln Pro Gly Gly Asp Thr Ile Ser Ser Cys Pro Ser Ala Ser Ala
        285                 290                 295

TAC GGC GGC CTC GCC ACC ATG GGC AAG GCC CTG AGC AGC GGC ATG GTG     1012
Tyr Gly Gly Leu Ala Thr Met Gly Lys Ala Leu Ser Ser Gly Met Val
    300                 305                 310

CTC GTG TTC AGC ATT TGG AAC GAC AAC AGC CAG TAC ATG AAC TGG CTC     1060
Leu Val Phe Ser Ile Trp Asn Asp Asn Ser Gln Tyr Met Asn Trp Leu
315                 320                 325                 330

GAC AGC GGC AAC GCC GGC CCC TGC AGC AGC ACC GAG GGC AAC CCA TCC     1108
Asp Ser Gly Asn Ala Gly Pro Cys Ser Ser Thr Glu Gly Asn Pro Ser
                335                 340                 345

AAC ATC CTG GCC AAC AAC CCC AAC ACG CAC GTC GTC TTC TCC AAC ATC     1156
```

```
Asn Ile Leu Ala Asn Asn Pro Asn Thr His Val Val Phe Ser Asn Ile
          350                 355                 360

CGC TGG GGA GAC ATT GGG TCT ACT ACG AAC TCG ACT GCG CCC CCG          1201
Arg Trp Gly Asp Ile Gly Ser Thr Thr Asn Ser Thr Ala Pro Pro
      365                 370                 375
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Gln Pro Gly Thr Ser Thr Pro Glu Val His Pro Lys Leu Thr Thr
 1               5                  10                  15

Tyr Lys Cys Thr Lys Ser Gly Gly Cys Val Ala Gln Asp Thr Ser Val
            20                  25                  30

Val Leu Asp Trp Asn Tyr Arg Trp Met His Asp Ala Asn Tyr Asn Ser
        35                  40                  45

Cys Thr Val Asn Gly Gly Val Asn Thr Thr Leu Cys Pro Asp Glu Ala
    50                  55                  60

Thr Cys Gly Lys Asn Cys Phe Ile Glu Gly Val Asp Tyr Ala Ala Ser
65                  70                  75                  80

Gly Val Thr Thr Ser Gly Ser Ser Leu Thr Met Asn Gln Tyr Met Pro
                85                  90                  95

Ser Ser Ser Gly Gly Tyr Ser Ser Val Ser Pro Arg Leu Tyr Leu Leu
            100                 105                 110

Asp Ser Asp Gly Glu Tyr Val Met Leu Lys Leu Asn Gly Gln Glu Leu
        115                 120                 125

Ser Phe Asp Val Asp Leu Ser Ala Leu Pro Cys Gly Glu Asn Gly Ser
    130                 135                 140

Leu Tyr Leu Ser Gln Met Asp Glu Asn Gly Gly Ala Asn Gln Tyr Asn
145                 150                 155                 160

Thr Ala Gly Ala Asn Tyr Gly Ser Gly Tyr Cys Asp Ala Gln Cys Pro
                165                 170                 175

Val Gln Thr Trp Arg Asn Gly Thr Leu Asn Thr Ser His Gln Gly Phe
            180                 185                 190

Cys Cys Asn Glu Met Asp Ile Leu Glu Gly Asn Ser Arg Ala Asn Ala
        195                 200                 205

Leu Thr Pro His Ser Cys Thr Ala Thr Ala Cys Asp Ser Ala Gly Cys
    210                 215                 220

Gly Phe Asn Pro Tyr Gly Ser Gly Tyr Lys Ser Tyr Tyr Gly Pro Gly
225                 230                 235                 240

Asp Thr Val Asp Thr Ser Lys Thr Phe Thr Ile Ile Thr Gln Phe Asn
                245                 250                 255
```

```
Thr Asp Asn Gly Ser Pro Ser Gly Asn Leu Val Ser Ile Thr Arg Lys
          260                 265                 270

Tyr Gln Gln Asn Gly Val Asp Ile Pro Ser Ala Gln Pro Gly Gly Asp
      275                 280                 285

Thr Ile Ser Ser Cys Pro Ser Ala Ser Ala Tyr Gly Gly Leu Ala Thr
  290                 295                 300

Met Gly Lys Ala Leu Ser Ser Gly Met Val Leu Val Phe Ser Ile Trp
305                 310                 315                 320

Asn Asp Asn Ser Gln Tyr Met Asn Trp Leu Asp Ser Gly Asn Ala Gly
                325                 330                 335

Pro Cys Ser Ser Thr Glu Gly Asn Pro Ser Asn Ile Leu Ala Asn Asn
          340                 345                 350

Pro Asn Thr His Val Val Phe Ser Asn Ile Arg Trp Gly Asp Ile Gly
      355                 360                 365

Ser Thr Thr Asn Ser Thr Ala Pro Pro
  370                 375
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..56, 231..1155)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
GGG GTC CGA TTT GCC GGC GTT AAC ATC GCG GGT TTT GAC TTT GGC TGT        48
Gly Val Arg Phe Ala Gly Val Asn Ile Ala Gly Phe Asp Phe Gly Cys
  1               5                  10                  15

ACC ACA  GA  GTGAGTACCC TTGTTTCCTG GTGTTGCTGG CTGGTTGGGC              96
Thr Thr  Asp

GGGTATACAG CGAAGCGGAC GCAAGAACAC CGCCGGTCCG CCACCATCAA GATGTGGGTG     156

GTAAGCGGCG GTGTTTTGTA CAACTACCTG ACAGCTCACT CAGGAAATGA GAATTAATGG     216

AAGTCTTGTT ACAG T GGC ACT TGC GTT ACC TCG AAG GTT TAT CCT CCG          264
                  Gly Thr Cys Val Thr Ser Lys Val Tyr Pro Pro
                   20                  25                  30

TTG AAG AAC TTC ACC GGC TCA AAC AAC TAC CCC GAT GGC ATC GGC CAG       312
Leu Lys Asn Phe Thr Gly Ser Asn Asn Tyr Pro Asp Gly Ile Gly Gln
                35                  40                  45

ATG CAG CAC TTC GTC AAC GAG GAC GGG ATG ACT ATT TTC CGC TTA CCT       360
Met Gln His Phe Val Asn Glu Asp Gly Met Thr Ile Phe Arg Leu Pro
            50                  55                  60
```

```
GTC GGA TGG CAG TAC CTC GTC AAC AAC AAT TTG GGC GGC AAT CTT GAT      408
Val Gly Trp Gln Tyr Leu Val Asn Asn Asn Leu Gly Gly Asn Leu Asp
        65                  70                  75

TCC ACG AGC ATT TCC AAG TAT GAT CAG CTT GTT CAG GGG TGC CTG TCT      456
Ser Thr Ser Ile Ser Lys Tyr Asp Gln Leu Val Gln Gly Cys Leu Ser
    80                  85                  90

CTG GGC GCA TAC TGC ATC GTC GAC ATC CAC AAT TAT GCT CGA TGG AAC      504
Leu Gly Ala Tyr Cys Ile Val Asp Ile His Asn Tyr Ala Arg Trp Asn
 95                 100                 105                 110

GGT GGG ATC ATT GGT CAG GGC GGC CCT ACT AAT GCT CAA TTC ACG AGC      552
Gly Gly Ile Ile Gly Gln Gly Gly Pro Thr Asn Ala Gln Phe Thr Ser
                115                 120                 125

CTT TGG TCG CAG TTG GCA TCA AAG TAC GCA TCT CAG TCG AGG GTG TGG      600
Leu Trp Ser Gln Leu Ala Ser Lys Tyr Ala Ser Gln Ser Arg Val Trp
            130                 135                 140

TTC GGC ATC ATG AAT GAG CCC CAC GAC GTG AAC ATC AAC ACC TGG GCT      648
Phe Gly Ile Met Asn Glu Pro His Asp Val Asn Ile Asn Thr Trp Ala
        145                 150                 155

GCC ACG GTC CAA GAG GTT GTA ACC GCA ATC CGC AAC GCT GGT GCT ACG      696
Ala Thr Val Gln Glu Val Val Thr Ala Ile Arg Asn Ala Gly Ala Thr
    160                 165                 170

TCG CAA TTC ATC TCT TTG CCT GGA AAT GAT TGG CAA TCT GCT GGG GCT      744
Ser Gln Phe Ile Ser Leu Pro Gly Asn Asp Trp Gln Ser Ala Gly Ala
175                 180                 185                 190

TTC ATA TCC GAT GGC AGT GCA GCC GCC CTG TCT CAA GTC ACG AAC CCG      792
Phe Ile Ser Asp Gly Ser Ala Ala Ala Leu Ser Gln Val Thr Asn Pro
                195                 200                 205

GAT GGG TCA ACA ACG AAT CTG ATT TTT GAC GTG CAC AAA TAC TTG GAC      840
Asp Gly Ser Thr Thr Asn Leu Ile Phe Asp Val His Lys Tyr Leu Asp
            210                 215                 220

TCA GAC AAC TCC GGT ACT CAC GCC GAA TGT ACT ACA AAT AAC ATT GAC      888
Ser Asp Asn Ser Gly Thr His Ala Glu Cys Thr Thr Asn Asn Ile Asp
        225                 230                 235

GGC GCC TTT TCT CCG CTT GCC ACT TGG CTC CGA CAG AAC AAT CGC CAG      936
Gly Ala Phe Ser Pro Leu Ala Thr Trp Leu Arg Gln Asn Asn Arg Gln
    240                 245                 250

GCT ATC CTG ACA GAA ACC GGT GGT GGC AAC GTT CAG TCC TGC ATA CAA      984
Ala Ile Leu Thr Glu Thr Gly Gly Gly Asn Val Gln Ser Cys Ile Gln
255                 260                 265                 270

GAC ATG TGC CAG CAA ATC CAA TAT CTC AAC CAG AAC TCA GAT GTC TAT     1032
Asp Met Cys Gln Gln Ile Gln Tyr Leu Asn Gln Asn Ser Asp Val Tyr
                275                 280                 285

CTT GGC TAT GTT GGT TGG GGT GCC GGA TCA TTT GAT AGC ACG TAT GTC     1080
Leu Gly Tyr Val Gly Trp Gly Ala Gly Ser Phe Asp Ser Thr Tyr Val
            290                 295                 300

CTG ACG GAA ACA CCG ACT AGC AGT GGT AAC TCA TGG ACG GAC ACA TCC     1128
Leu Thr Glu Thr Pro Thr Ser Ser Gly Asn Ser Trp Thr Asp Thr Ser
        305                 310                 315

TTG GTC AGC TCG TGT CTC GCA AGA AAG                                1155
Leu Val Ser Ser Cys Leu Ala Arg Lys
    320                 325
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Gly Val Arg Phe Ala Gly Val Asn Ile Ala Gly Phe Asp Phe Gly Cys
 1               5                  10                  15

Thr Thr Asp Gly Thr Cys Val Thr Ser Lys Val Tyr Pro Pro Leu Lys
            20                  25                  30

Asn Phe Thr Gly Ser Asn Asn Tyr Pro Asp Gly Ile Gly Gln Met Gln
        35                  40                  45

His Phe Val Asn Glu Asp Gly Met Thr Ile Phe Arg Leu Pro Val Gly
    50                  55                  60

Trp Gln Tyr Leu Val Asn Asn Asn Leu Gly Gly Asn Leu Asp Ser Thr
65                  70                  75                  80

Ser Ile Ser Lys Tyr Asp Gln Leu Val Gln Gly Cys Leu Ser Leu Gly
                85                  90                  95

Ala Tyr Cys Ile Val Asp Ile His Asn Tyr Ala Arg Trp Asn Gly Gly
            100                 105                 110

Ile Ile Gly Gln Gly Gly Pro Thr Asn Ala Gln Phe Thr Ser Leu Trp
        115                 120                 125

Ser Gln Leu Ala Ser Lys Tyr Ala Ser Gln Ser Arg Val Trp Phe Gly
    130                 135                 140

Ile Met Asn Glu Pro His Asp Val Asn Ile Asn Thr Trp Ala Ala Thr
145                 150                 155                 160

Val Gln Glu Val Val Thr Ala Ile Arg Asn Ala Gly Ala Thr Ser Gln
                165                 170                 175

Phe Ile Ser Leu Pro Gly Asn Asp Trp Gln Ser Ala Gly Ala Phe Ile
            180                 185                 190

Ser Asp Gly Ser Ala Ala Ala Leu Ser Gln Val Thr Asn Pro Asp Gly
        195                 200                 205

Ser Thr Thr Asn Leu Ile Phe Asp Val His Lys Tyr Leu Asp Ser Asp
    210                 215                 220

Asn Ser Gly Thr His Ala Glu Cys Thr Thr Asn Asn Ile Asp Gly Ala
225                 230                 235                 240

Phe Ser Pro Leu Ala Thr Trp Leu Arg Gln Asn Asn Arg Gln Ala Ile
                245                 250                 255

Leu Thr Glu Thr Gly Gly Gly Asn Val Gln Ser Cys Ile Gln Asp Met
            260                 265                 270

Cys Gln Gln Ile Gln Tyr Leu Asn Gln Asn Ser Asp Val Tyr Leu Gly
        275                 280                 285

Tyr Val Gly Trp Gly Ala Gly Ser Phe Asp Ser Thr Tyr Val Leu Thr
```

290 295 300

Glu Thr Pro Thr Ser Ser Gly Asn Ser Trp Thr Asp Thr Ser Leu Val
305 310 315 320

Ser Ser Cys Leu Ala Arg Lys
325

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..72

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGT GGC ACC ACC ACC ACC CGC CGC CCA GCC ACT ACC ACT GGA AGC TCT 48
Arg Gly Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser
1 5 10 15

CCC GGA CCT ACC CAG TCT CAC TAC 72
Pro Gly Pro Thr Gln Ser His Tyr
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Arg Gly Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser
 1               5                  10                  15

Pro Gly Pro Thr Gln Ser His Tyr
            20
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..129

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
GGC GCT GCA AGC TCA AGC TCG TCC ACG CGC GCC GCG TCG ACG ACT TCT        48
Gly Ala Ala Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser
 1               5                  10                  15

CGA GTA TCC CCC ACA ACA TCC CGG TCG AGC TCC GCG ACG CCT CCA CCT        96
Arg Val Ser Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro
            20                  25                  30

GGT TCT ACT ACT ACC AGA GTA CCT CCA GTC GGA                          129
Gly Ser Thr Thr Thr Arg Val Pro Pro Val Gly
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Gly Ala Ala Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser
 1               5                  10                  15

Arg Val Ser Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro
            20                  25                  30

Gly Ser Thr Thr Thr Arg Val Pro Pro Val Gly
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..81

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
CCC CCG CCT GCG TCC AGC ACG ACG TTT TCG ACT ACA CCG AGG AGC TCG        48
Pro Pro Pro Ala Ser Ser Thr Thr Phe Ser Thr Thr Pro Arg Ser Ser
 1               5                  10                  15

ACG ACT TCG AGC AGC CCG AGC TGC ACG CAG ACT                        81
Thr Thr Ser Ser Ser Pro Ser Cys Thr Gln Thr
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Pro Pro Pro Ala Ser Ser Thr Thr Phe Ser Thr Thr Pro Arg Ser Ser
 1               5                  10                  15

Thr Thr Ser Ser Ser Pro Ser Cys Thr Gln Thr
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
CCG GGA GCC ACT ACT ATC ACC ACT TCG ACC CGG CCA CCA TCC GGT CCA        48
Pro Gly Ala Thr Thr Ile Thr Thr Ser Thr Arg Pro Pro Ser Gly Pro
  1               5                  10                  15

ACC ACC ACC ACC AGG GCT ACC TCA ACA AGC TCA TCA ACT CCA CCC ACG        96
Thr Thr Thr Thr Arg Ala Thr Ser Thr Ser Ser Ser Thr Pro Pro Thr
            20                  25                  30

AGC TCT                                                               102
Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Pro Gly Ala Thr Thr Ile Thr Thr Ser Thr Arg Pro Pro Ser Gly Pro
  1               5                  10                  15

Thr Thr Thr Thr Arg Ala Thr Ser Thr Ser Ser Ser Thr Pro Pro Thr
            20                  25                  30

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATG TAT CGG AAG TTG GCC GTC ATC TCG GCC TTC TTG GCC ACA GCT CGT        48
Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr Ala Arg
  1               5                  10                  15

GCT                                                                   51
Ala
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr Ala Arg
 1               5                  10                  15

Ala
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..72

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
ATG ATT GTC GGC ATT CTC ACC ACG CTG GCT ACG CTG GCC ACA CTC GCA      48
Met Ile Val Gly Ile Leu Thr Thr Leu Ala Thr Leu Ala Thr Leu Ala
 1               5                  10                  15

GCT AGT GTG CCT CTA GAG GAG CGG                                     72
Ala Ser Val Pro Leu Glu Glu Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Ile Val Gly Ile Leu Thr Thr Leu Ala Thr Leu Ala Thr Leu Ala
 1               5                  10                  15

Ala Ser Val Pro Leu Glu Glu Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
ATG GCG CCC TCA GTT ACA CTG CCG TTG ACC ACG GCC ATC CTG GCC ATT        48
Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile
  1               5                  10                  15

GCC CGG CTC GTC GCC GCC                                            66
Ala Arg Leu Val Ala Ala
            20
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile
  1               5                  10                  15

Ala Arg Leu Val Ala Ala
            20
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..63

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
ATG AAC AAG TCC GTG GCT CCA TTG CTG CTT GCA GCG TCC ATA CTA TAT        48
Met Asn Lys Ser Val Ala Pro Leu Leu Leu Ala Ala Ser Ile Leu Tyr
  1               5                  10                  15

GGC GGC GCC GTC GCA                                                63
Gly Gly Ala Val Ala
            20
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Asn Lys Ser Val Ala Pro Leu Leu Leu Ala Ala Ser Ile Leu Tyr
1 5 10 15

Gly Gly Ala Val Ala
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 777 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAACCAGCTG TGACCAGTGG GCAACCTTCA CTGGCAACGG CTACACAGTC AGCAACAACC 60

TTTGGGGAGC ATCAGCCGGC TCTGGATTTG GCTGCGTGAC GGCGGTATCG CTCAGCGGCG 120

GGGCCTCCTG GCACGCAGAC TGGCAGTGGT CCGGCGGCCA GAACAACGTC AAGTCGTACC 180

AGAACTCTCA GATTGCCATT CCCCAGAAGA GGACCGTCAA CAGCATCAGC AGCATGCCCA 240

CCACTGCCAG CTGGAGCTAC AGCGGGAGCA ACATCCGCGC TAATGTTGCG TATGACTTGT 300

TCACCGCAGC CAACCCGAAT CATGTCACGT ACTCGGGAGA CTACGAACTC ATGATCTGGT 360

AAGCCATAAG AAGTGACCCT CCTTGATAGT TTCGACTAAC AACATGTCTT GAGGCTTGGC 420

AAATACGGCG ATATTGGGCC GATTGGGTCC TCACAGGGAA CAGTCAACGT CGGTGGCCAG 480

AGCTGGACGC TCTACTATGG CTACAACGGA GCCATGCAAG TCTATTCCTT TGTGGCCCAG 540

ACCAACACTA CCAACTACAG CGGAGATGTC AAGAACTTCT TCAATTATCT CCGAGACAAT 600

AAAGGATACA ACGCTGCAGG CCAATATGTT CTTAGTAAGT CACCCTCACT GTGACTGGGC 660

TGAGTTTGTT GCAACGTTTG CTAACAAAAC CTTCGTATAG GCTACCAATT TGGTACCGAG 720

CCCTTCACGG GCAGTGGAAC TCTGAACGTC GCATCCTGGA CCGCATCTAT CAACTAA 777

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 218 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Gln Thr Ser Cys Asp Gln Trp Ala Thr Phe Thr Gly Asn Gly Tyr Thr
1               5                  10                  15

Val Ser Asn Asn Leu Trp Gly Ala Ser Ala Gly Ser Gly Phe Gly Cys
            20                  25                  30

Val Thr Ala Val Ser Leu Ser Gly Gly Ala Ser Trp His Ala Asp Trp
        35                  40                  45

Gln Trp Ser Gly Gly Gln Asn Asn Val Lys Ser Tyr Gln Asn Ser Gln
    50                  55                  60

Ile Ala Ile Pro Gln Lys Arg Thr Val Asn Ser Ile Ser Ser Met Pro
65                  70                  75                  80

Thr Thr Ala Ser Trp Ser Tyr Ser Gly Ser Asn Ile Arg Ala Asn Val
                85                  90                  95

Ala Tyr Asp Leu Phe Thr Ala Ala Asn Pro Asn His Val Thr Tyr Ser
            100                 105                 110

Gly Asp Tyr Glu Leu Met Ile Trp Leu Gly Lys Tyr Gly Asp Ile Gly
        115                 120                 125

Pro Ile Gly Ser Ser Gln Gly Thr Val Asn Val Gly Gly Gln Ser Trp
    130                 135                 140

Thr Leu Tyr Tyr Gly Tyr Asn Gly Ala Met Gln Val Tyr Ser Phe Val
145                 150                 155                 160

Ala Gln Thr Asn Thr Thr Asn Tyr Ser Gly Asp Val Lys Asn Phe Phe
                165                 170                 175

Asn Tyr Leu Arg Asp Asn Lys Gly Tyr Asn Ala Ala Gly Gln Tyr Val
            180                 185                 190

Leu Ser Tyr Gln Phe Gly Thr Glu Pro Phe Thr Gly Ser Gly Thr Leu
        195                 200                 205

Asn Val Ala Ser Trp Thr Ala Ser Ile Asn
    210                 215
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
ATGAAGTTCC TTCAAGTCCT CCCTGCCCTC ATACCGGCCG CCCTGGCC                48
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Lys Phe Leu Gln Val Leu Pro Ala Leu Ile Pro Ala Ala Leu Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AGCTCGTAGA GCGTTGACTT GCCTGTGGTC TGTCCAGACG GGGGACGATA GAATGCG 57

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GTCACCTTCT CCAACATCAA GTTCGGACCC ATTGGCAGCA CCGGCTAA 48

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGGGTTTAAA CCCGCGGGGA TT 22

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGAGCCGAGG CCTCC 15

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGCTTGAGAT CTGAAGCT 18

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GATCGC 6

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TTATTAGTAA TATGCA 16

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTAGAGGAGC GGTCGGGAAC CGCTAC 26

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Leu Glu Glu Arg Ser Gly Thr Ala Thr
1 5

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

AAACCCCGGG TGATTTATTT TTTTTGTATC TACTTCTGA 39

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Lys Pro Arg Val Ile Tyr Phe Phe Cys Ile Tyr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Cys Gly Gly Gln Asn Val Ser Gly Pro Thr Cys Cys Ala Ser Gly Ser
1 5 10 15

Thr Cys

Claims:

1. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising a CBHI catalytic core protein or derivatives thereof which exhibit exoglucanase activity.

2. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising a CBHII catalytic core protein or derivatives thereof which exhibit exoglucanase activity.

3. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising an EGI catalytic core protein or derivatives thereof which exhibit endoglucanase activity.

4. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising an EGII catalytic core protein or derivatives thereof which exhibit endoglucanase activity.

5. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising the cellulose binding domain derived from CBHI or derivatives thereof which exhibit cellulose binding.

6. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising the cellulose binding domain derived from CBHII or derivatives thereof which exhibit cellulose binding.

7. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising the cellulose binding domain derived from EGI or derivatives thereof which exhibit cellulose binding.

8. A substantially pure truncated fungal cellulase protein derived from Trichoderma comprising the cellulose binding domain derived from EGII or derivatives thereof which exhibit cellulose binding.

9. The truncated fungal cellulase protein according to claim 1-9 in the alternative wherein said Trichoderma is Trichoderma longibrachiatum.

10. The truncated fungal cellulase of claim 1 wherein said CBHI catalytic core consists essentially of the amino acid sequence set forth in SEQ ID:NO 1 and derivatives thereof.

11. The truncated fungal cellulase of claim 2 wherein said CBHII catalytic core consists essentially of the amino acid sequence set forth in SEQ ID:NO 2 and derivatives thereof.

12. The truncated fungal cellulase of claim 3 wherein said EGI catalytic core consists essentially of the amino acid sequence set forth in SEQ ID:NO 3 and derivatives thereof.

13. The truncated fungal cellulase of claim 4 wherein said EGII catalytic core consists essentially of the amino acid sequence set forth in SEQ ID:NO 4 and derivatives thereof.

14. The truncated fungal cellulase of claim 5 wherein said CBHI cellulose binding domain consists essentially of the amino acid sequence set forth in SEQ:ID NO 5 and derivatives thereof.

15. The truncated fungal cellulase of claim 6 wherein said CBHII cellulose binding domain consists essentially of

the amino acid sequence set forth in SEQ ID:NO 6 and derivatives thereof.

16. The truncated fungal cellulase of claim 7 wherein said EGI cellulose binding domain consists essentially of the amino acid sequence set forth in SEQ ID:NO 7 and derivatives thereof.

17. The truncated fungal cellulase of claim 8 wherein said EGII cellulose binding domain consists essentially of the amino acid sequence set forth in SEQ ID:NO 8 and derivatives thereof.

18. A DNA gene fragment or variant thereof derived from Trichoderma which codes for CBHI catalytic core or derivatives thereof which exhibit exoglucanase activity.

19. The DNA fragment of claim 18 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for CBHI catalytic core.

20. The DNA gene fragment of claim 19 further comprising a DNA sequence or portion thereof derived from CBHI binding domain which does not code for a protein that exhibits cellulose binding.

21. The DNA gene fragment of claim 18 wherein said DNA sequence coding for the CBHI catalytic core is set forth in SEQ ID:NO 9.

22. The DNA gene fragment of claim 19 wherein said DNA fragment coding for the CBHI catalytic core is set forth in SEQ ID:NO 9 and the said hinge region DNA sequence is set forth in SEQ ID:NO 17.

23. The DNA gene fragment of claim 20 wherein said DNA fragment coding for the CBHI catalytic core is set forth in

SEQ ID:NO 9, said hinge region DNA sequence is set forth in SEQ ID:NO 17 and said CBHI binding domain is set forth in SEQ ID:NO 13.

24. A DNA gene fragment or variants thereof derived from Trichoderma which codes for CBHII catalytic core or derivatives thereof which exhibit exoglucanase activity.

25. The DNA fragment of claim 24 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for CBHII catalytic core.

26. The DNA gene fragment of claim 25 further comprising a DNA sequence or portion thereof derived from CBHII binding domain which does not code for a protein that exhibits cellulose binding.

27. The DNA gene fragment of claim 24 wherein said DNA sequence coding for the CBHII catalytic core is set forth in SEQ ID:NO 10.

28. The DNA gene fragment of claim 25 wherein said DNA fragment coding for the CBHII catalytic core is set forth in SEQ ID:NO 10 and said hinge region DNA sequence is set forth in SEQ ID:NO 18.

29. The DNA gene fragment of claim 26 wherein said DNA fragment coding for the CBHII catalytic core is set forth in SEQ ID:NO 10, said hinge region DNA sequence is set forth in SEQ ID:NO 18 and said CBHII binding domain is set forth in SEQ ID:NO 14.

30. A DNA gene fragment or variants thereof derived from Trichoderma which codes for EGI catalytic core or derivatives thereof which exhibit endoglucanase activity.

31. The DNA fragment of claim 30 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for EGI catalytic core.

32. The DNA gene fragment of claim 31 further comprising a DNA sequence or portion thereof derived from EGI binding domain which does not code for a protein that exhibits cellulose binding.

33. The DNA gene fragment of claim 30 wherein said DNA sequence coding for the EGI catalytic core is set forth in SEQ ID:NO 11.

34. The DNA gene fragment of claim 31 wherein said DNA fragment coding for the EGI catalytic core is set forth in SEQ ID:NO 11 and said hinge region DNA sequence is set forth in SEQ ID:NO 19.

35. The DNA gene fragment of claim 32 wherein said DNA fragment coding for the EGI catalytic core is set forth in SEQ ID:NO 11, said hinge region DNA sequence is set forth in SEQ ID:NO 19 and said EGI binding domain is set forth in SEQ ID:NO 15.

36. A DNA gene fragment or variants derived from Trichoderma which codes for EGII catalytic core or derivatives thereof which exhibit endoglucanase activity.

37. The DNA fragment of claim 36 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for EGII catalytic core.

38. The DNA gene fragment of claim 37 further comprising a DNA sequence or portion thereof derived from EGII binding domain which does not code for a protein that exhibits cellulose binding.

39. The DNA gene fragment of claim 36 wherein said DNA sequence coding for the EGII catalytic core is set forth in SEQ ID:NO 12.

40. The DNA gene fragment of claim 37 wherein said DNA fragment coding for the EGII catalytic core is set forth in SEQ ID:NO 12 and said hinge region DNA sequence is set forth in SEQ ID:NO 20.

41. The DNA gene fragment of claim 38 wherein said DNA fragment coding for the EGII catalytic core is set forth in SEQ ID:NO 12, said hinge region DNA sequence is set forth in SEQ ID:NO 20 and said EGII binding domain is set forth in SEQ ID:NO 16.

42. A DNA gene fragment or variants thereof derived from Trichoderma which codes for the CBHI binding domain or derivatives thereof which exhibit cellulose binding.

43. The DNA fragment of claim 42 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for the CBHI binding domain.

44. The DNA gene fragment of claim 43 further comprising a DNA sequence or portion thereof derived from the CBHI catalytic core domain which does not code for a protein that exhibits exoglucanase activity.

45. The DNA gene fragment of claim 42 wherein said DNA sequence coding for the CBHI binding domain is set forth in SEQ ID:NO 13.

46. The DNA gene fragment of claim 43 wherein said DNA fragment coding for the CBHI binding domain is set forth in SEQ ID:NO 13 and said hinge region DNA sequence is set forth in SEQ ID:NO 17.

47. The DNA gene fragment of claim 44 wherein said DNA fragment coding for the CBHI binding domain is set forth in SEQ ID:NO 13, said hinge region DNA sequence is set forth in SEQ ID:NO 17 and said CBHI core domain is set forth in SEQ ID:NO 9.

48. A DNA gene fragment or variants thereof derived from Trichoderma which codes for the CBHII binding domain or derivatives thereof which exhibit cellulose binding.

49. The DNA fragment of claim 48 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for the CBHII binding domain.

50. The DNA gene fragment of claim 49 further comprising a DNA sequence or portion thereof derived from the CBHII catalytic core domain which does not code for a protein that exhibits exoglucanase activity.

51. The DNA gene fragment of claim 48 wherein said DNA sequence coding for the CBHII binding domain is set forth in SEQ ID:NO 14.

52. The DNA gene fragment of claim 49 wherein said DNA fragment coding for the CBHII binding domain is set forth in SEQ ID:NO 14 and said hinge region DNA sequence is set forth in SEQ ID:NO 18.

53. The DNA gene fragment of claim 50 wherein said DNA fragment coding for the CBHII binding domain is set forth in SEQ ID:NO 14 and said hinge region DNA sequence is set forth in SEQ ID:NO 18.

54. A DNA gene fragment or variants thereof derived from Trichoderma which codes for the EGI binding domain or derivatives thereof which exhibit cellulose binding.

55. The DNA fragment of claim 54 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for the EGI binding domain.

56. The DNA gene fragment of claim 55 further comprising a DNA sequence or portion thereof derived from the EGI catalytic core domain which does not code for a protein that exhibits endoglucanase activity.

57. The DNA gene fragment of claim 54 wherein said DNA sequence coding for the EGI binding domain is set forth in SEQ ID:NO 15.

58. The DNA gene fragment of claim 55 wherein said DNA fragment coding for the EGI binding domain is set forth in SEQ ID:NO 15 and said hinge region DNA sequence is set forth in SEQ ID:NO 19.

59. The DNA gene fragment of claim 56 wherein said DNA fragment coding for the EGI binding domain is set forth in SEQ ID:NO 15, said hinge region DNA sequence is set forth in SEQ ID:NO 19 and said EGI core domain is set forth in SEQ ID:NO 11.

60. A DNA gene fragment or variants thereof derived from Trichoderma which codes for the EGII binding domain or derivatives thereof which exhibit cellulose binding.

61. The DNA fragment of claim 60 further comprising a hinge region DNA sequence or portion thereof operably linked to said fragment coding for the EGII binding domain.

62. The DNA gene fragment of claim 61 further comprising a DNA sequence or portion thereof derived from the EGII catalytic core domain which does not code for a protein that exhibits endoglucanase activity.

63. The DNA gene fragment of claim 60 wherein said DNA sequence coding for the EGII binding domain is set forth in SEQ ID:NO 16.

64. The DNA gene fragment of claim 61 wherein said DNA fragment coding for the EGII binding domain is set forth in SEQ ID:NO 16 and said hinge region DNA sequence is set forth in SEQ ID:NO 20.

65. The DNA gene fragment of claim 62 wherein said DNA fragment coding for the EGII binding domain is set forth in SEQ ID:NO 16, said hinge region DNA sequence is set forth in SEQ ID:NO 20 and said EGII core domain is set forth in SEQ ID:NO 12.

66. An expression vector called pTEX having the accession #---.

67. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase, said DNA gene fragment is operably linked to one or more regulatory DNA sequences in said vector.

68. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase and a selectable marker.

69. The expression vector according to claim 67 wherein said one or more regulatory DNA sequences codes a functionally active promoter and terminator.

70. The expression vector according to claim 67 wherein said at least one truncated DNA gene fragment or variant thereof carries a signal sequence and said one or more

regulatory DNA sequences codes a functionally active promotor and terminator.

71. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 21, 22 or 23.

72. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 27, 28 or 29.

73. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 33, 34 or 35.

74. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 39, 40 or 41.

75. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 45, 46 or 47.

76. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 51, 52 or 53.

77. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 57, 58 or 59.

78. An expression vector constructed from Trichoderma which carries at least one truncated DNA gene fragment or variant thereof from a Trichoderma cellulase operably linked to one or more regulatory DNA sequences in said vector and a selectable marker, said truncated DNA fragment is derived from claim 63, 64 or 65.

79. A transformed fungal cell comprising an expression vector comprising a DNA fragment or variant thereof encoding a truncated cellulase enzyme or derivative thereof derived from Trichoderma with catalytic core activity operably linked to one or more regulatory DNA sequences and a selectable marker.

80. The transformed fungal cell according to claim 79 wherein said DNA fragment codes for CBHI catalytic core or derivatives thereof which exhibit exoglucanase activity.

81. The transformed fungal cell according to claim 79 wherein said DNA fragment codes for CBHII catalytic core or derivatives thereof which exhibit exoglucanase activity.

82. The transformed fungal cell according to claim 79 wherein said DNA fragment codes for EGI catalytic core or derivatives thereof which exhibit endoglucanase activity.

83. The transformed fungal cell according to claim 79 wherein said DNA fragment codes for EGII catalytic core or derivatives thereof which exhibit endoglucanase activity.

84. A transformed fungal cell comprising an expression vector comprising a DNA fragment or variant thereof encoding a truncated cellulase enzyme or derivative thereof derived from Trichoderma with cellulose binding properties operably linked to one or more regulatory DNA sequences and a selectable marker.

85. The transformed fungal cell according to claim 84 wherein said DNA fragment codes for CBHI cellulose binding domain or derivatives thereof which exhibit cellulose binding.

86. The transformed fungal cell according to claim 84 wherein said DNA fragment codes for CBHII cellulose binding domain or derivatives thereof which exhibit cellulose binding.

87. The transformed fungal cell according to claim 84 wherein said DNA fragment codes for EGI cellulose binding domain or derivatives thereof which exhibit cellulose binding.

88. The transformed fungal cell according to claim 84 wherein said DNA fragment codes for EGII cellulose binding domain or derivatives thereof which exhibit cellulose binding.

89. A process for transforming a Trichoderma host cell such that said host cell is capable of expressing one or more functional active truncated cellulases, comprising the steps of:

a) obtaining a Trichoderma host cell which is missing one or more cellulase activities;

b) treating said cell with one or more DNA vectors, said DNA vector comprising one or more truncated cellulase DNA fragments or cellulase DNA fragment variants operatively linked to a regulatory DNA sequence under conditions such that said one or more DNA constructs integrate into the genome of said cell and transformed cells are effectuated; and

c) isolating said transformed cells from non-transformed cells.

90. The process according to Claim 89 wherein the fungal host cell is Trichoderma longibrachiatum.

91. The process according to Claim 89 wherein said one or more DNA vectors comprises a predetermined selectable marker gene.

92. The process according to Claim 91 wherein the selectable marker gene is selected from the group consisting of pyr4, argB, trpC and amdS.

93. The process according to Claim 89 wherein said cellulase DNA fragments encode for a truncated cellulase with exocellobiohydrolase activity or endoglucanase activity.

94. The process according to Claim 93 wherein said truncated cellulase DNA fragments is selected from the group consisting of CBHI, CBHII, EGI, EG II, EGIII or EGV.

95. The transformed fungal cell according to claim 79 wherein said DNA fragment is a variant DNA fragment that codes for EGIII catalytic core derivatives thereof which exhibit cellulose binding.

```
AAGCTTAGCCAAGAACAATAGCCGATAAAGATAGCCTCATTAAACGGAAT
                                                    50
GAGCTAGTAGGCAAAGTCAGCGAATGTGTATATATAAAGGTTCGAGGTCC
                                                   100
GTGCCTCCCTCATGCTCTCCCCATCTACTCATCAACTCAGATCCTCCAGG
                                                   150
AGACTTGTACACCATNTTTTGAGGCACAGAAACCCAATAGTCAACCGCGG
                                                   200
ACTGGCATCATGTATCGGAAGTTGGCCGTCATCTCGGCCTTCTTGGCCAC
                                                   250
                  Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr
AGCTCGTGCTCAGTCGGCCTGCACTCTCCAATCGGAGACTCACCCGCCTC
                                                   300
  Ala Arg Ala Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro
TGACATGGCAGAAATGCTCGTCTGGTGGCACTTGCACTCAACAGACAGGC
                                                   350
Leu Thr Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly
TCCGTGGTCATCGACGCCAACTGGCGCTGGACTCACGCTACGAACAGCAG
                                                   400
 Ser Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
CACGAACTGCTACGATGGCAACACTTGGAGCTCGACCCTATGTCCTGACA
                                                   450
  Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
ACGAGACCTGCGCGAAGAACTGCTGTCTGGACGGTGCCGCCTACGCGTCC
                                                   500
Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala Ser
ACGTACGGAGTTACCACGAGCGGTAACAGCCTCTCCATTGGCTTTGTCAC
                                                   550
 Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe Val Thr
CCAGTCTGCGCAGAAGAACGTTGGCGCTCGCCTTTACCTTATGGCGAGCG
                                                   600
  Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met Ala Ser
ACACGACCTACCAGGAATTCACCTGCTTGGCAACGAGTTCTCTTTCGAT
                                                   650
Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe Ser Phe Asp
GTTGATGTTTCGCAGCTGCCGTAAGTGACTTACCATGAACCCCTGACGTA
                                                   700
 Val Asp Val Ser Gln Leu Pro
TCTTCTTGTGGGCTCCCAGCTGACTGGCCAATTTAAGGTGCGGCTTGAAC
                                                   750
                           Cys Gly Leu Asn
GGAGCTCTCTACTTCGTGTCCATGGACGCGGATGGTGGCGTGAGCAAGTA
                                                   800
 Gly Ala Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr
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FIG._1A

RECTIFIED SHEET (RULE 91)

ISA/EP

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TCCCACCAACACCGCTGGCGCCAAGTACGGCACGGGGTACTGTGACAGCC                  850
  Pro Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser
AGTGTCCCCGCGATCTGAAGTTCATCAATGGCCAGGCCAACGTTGAGGGC                  900
Gln Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
TGGGAGCCGTCATCCAACAACGCAAACACGGGCATTGGAGGACACGGAAG                  950
  Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly Ser
CTGCTGCTCTGAGATGGATATCTGGGAGGCCAACTCCATCTCCGAGGCTC                 1000
  Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu Ala
TTACCCCCCACCCTTGCACGACTGTCGGCCAGGAGATCTGCGAGGGTGAT                 1050
Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu Gly Asp
GGGTGCGGCGGAACTTACTCCGATAACAGATATGGCGGCACTTGCGATCC                 1100
  Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr Cys Asp Pro
CGATGGCTGCGACTGGAACCCATACCGCCTGGGCAACACCAGCTTCTACG                 1150
  Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr Ser Phe Tyr
GCCCTGGCTCAAGCTTTACCCTCGATACCACCAAGAAATTGACCGTTGTC                 1200
Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys Leu Thr Val Val
ACCCAGTTCGAGACGTCGGGTGCCATCAACCGATACTATGTCCAGAATGG                 1250
  Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr Tyr Val Gln Asn Gly
CGTCACTTTCCAGCAGCCCAACGCCGAGCTTGGTAGTTACTCTGGCAACG                 1300
  Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly Ser Tyr Ser Gly Asn
AGCTCAACGATGATTACTGCACAGCTGAGGAGGCAGAATTCGGCGGATCC                 1350
Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu Ala Glu Phe Gly Gly Ser
TCTTTCTCAGACAAGGGCGGCCTGACTCAGTTCAAGAAGGCTACCTCTGG                 1400
  Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln Phe Lys Lys Ala Thr Ser Gly
CGGCATGGTTCTGGTCATGAGTCTGTGGGATGATGTGAGTTTGATGGACA                 1450
  Gly Met Val Leu Val Met Ser Leu Trp Asp Asp ──────────
AACATGCGCGTTGACAAAGAGTCAAGCAGCTGACTGAGATGTTACAGTAC                 1500
──────────────────────────────────────────────────────Tyr
TACGCCAACATGCTGTGGCTGGACTCCACCTACCCGACAAACGAGACCTC                 1550
  Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr Asn Glu Thr Ser
```

FIG._1B

RECTIFIED SHEET (RULE 91)

ISA/EP

```
CTCCACACCCGGTGCCGTGCGCGGAAGCTGCTCCACCAGCTCCGGTGTCC
                                                    1600
  Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr Ser Ser Gly Val
CTGCTCAGGTCGAATCTCAGTCTCCCAACGCCAAGGTCACCTTCTCCAAC
                                                    1650
Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys Val Thr Phe Ser Asn
ATCAAGTTCGGACCCATTGGCAGCACCGGCAACCCTAGCGGCGGCAACCC
                                                    1700
 Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn Pro Ser Gly Gly Asn Pro
TCCCGGCGGAAACCGTGGCACCACCACCACCCGCCGCCCAGCCACTACCA
                                                    1750
  Pro Gly Gly Asn Arg Gly Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr
CTGGAAGCTCTCCCGGACCTACCCAGTCTCACTACGGCCAGTGCGGCGGT
                                                    1800
Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser His Tyr Gly Gln Cys Gly Gly
ATTGGCTACAGCGGCCCCACGGTCTGCGCCAGCGGCACAACTTGCCAGGT
                                                    1850
 Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser Gly Thr Thr Cys Gln Val
CCTGAACCCTTACTACTCTCAGTGCCTGTAAAGCTCCGTGCGAAAGCCTG
                                                    1900
  Leu Asn Pro Tyr Tyr Ser Gln Cys Leu •
ACGCACCGGTAGATTCTTGGTGAGCCCGTATCATGACGGCGGCGGGAGCT
                                                    1950
ACATGGCCCCGGGTGATTTATTTTTTTTGTATCTACTTCTGACCCTTTTC
                                                    2000
AAATATACGGTCAACTCATCTTTCACTGGAGATGCGGCCTGCTTGGTATT
                                                    2050
GCGATGTTGTCAGCTTGGCAAATTGTGGCTTTCGAAAACACAAAACGATT
                                                    2100
CCTTAGTAGCCATGCATTTTAAGATAACGGAATAGAAGAAAGAGGAAATT
                                                    2150
AAAAAAAAAAAAAAAACAAACATCCCGTTCATAACCCGTAGAATCGCCGC
                                                    2200
TCTTCGTGTATCCCAGTACCA
                    ------> 2221
```

FIG._1C

FIG._1A

FIG._1B

FIG._1C

FIG._1

```
GAATTCTAGGCTAGGTATGCGAGGCACGCGGATCTAGGGCAGACTGGGCA
                                                    50
TTGCATAGCTATGGTGTAGTAGAACTCCCGTCAACGGCTATTCTCACCTA
                                                   100
GACTTTCCCCTTCGAACTGACAAGTTGTTATATTGCCTGTGTACCAAGCG
                                                   150
CTAATGTGGACAGGATTAATGCCAGAGTTCATTAGCCTCAAGTAGAGCCT
                                                   200
ATTTCCTCGCCGGAAAGTCATCTCTCTTATTGCATTTCTGCCTTCCACTA
                                                   250
ACTCAGGGTGCAGCGCAACACTACACGCAACATATCACATTTATTAGCCG
                                                   300
TGCAACAAGGCTATTCTACGAAAAATGCTACACTCCACATGTTAAAGGCG
                                                   350
CATTCAACCAGCTTCTTTATTGGGTAATATACAGCCAGGCGGGGATGAAG
                                                   400
CTCATTAGCCGCCACTCAAGGCTATACAATGTTGCCAACTCTCCGGGCTT
                                                   450
TATCCTGTGCTCCCGAATACCACATCGTGATGATGCTTCAGCGCACGGAA
                                                   500
GTCACAGACACCGCCTGTATAAAAGGGGGACTGTGACCCTGTATGAGGCG
                                                   550
CAACATGGTCTCACAGCAGCTCACCTGAAGAGGCTTGTAAGATCACCCTC
                                                   600
TGTGTATTGCACCATGATTGTCGGCATTCTCACCACGCTGGCTACGCTGG
                                                   650
                    Met Ile Val Gly Ile Leu Thr Thr Leu Ala Thr Leu
CCACACTCGCAGCTAGTGTGCCTCTAGAGGAGCGGCAAGCTTGCTCAAGC
                                                   700
Ala Thr Leu Ala Ala Ser Val Pro Leu Glu Glu Arg Gln Ala Cys Ser Ser
GTCTGGTAATTATGTGAACCCTCTCAAGAGACCCAAATACTGAGATATGT
                                                   750
Val Trp
CAAGGGGCCAATGTGGTGGCCAGAATTGGTCGGGTCCGACTTGCTGTGCT
                                                   800
      Gly Gln Cys Gly Gly Gln Asn Trp Ser Gly Pro Thr Cys Cys Ala
TCCGGAAGCACATGCGTCTACTCCAACGACTATTACTCCCAGTGTCTTCC
                                                   850
Ser Gly Ser Thr Cys Val Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys Leu Pro
CGGCGCTGCAAGCTCAAGCTCGTCCACGCGCGCCGCGTCGACGACTTCTC
                                                   900
  Gly Ala Ala Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser
GAGTATCCCCCACAACATCCCGGTCGAGCTCCGCGACGCCTCCACCTGGT
                                                   950
Arg Val Ser Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro Gly
```

FIG._2A

```
TCTACTACTACCAGAGTACCTCCAGTCGGATCGGGAACCGCTACGTATTC
                                                    1000
Ser Thr Thr Thr Arg Val Pro Pro Val Gly Ser Gly Thr Ala Thr Tyr Ser
AGGCAACCCTTTTGTTGGGGTCACTCCTTGGGCCAATGCATATTACGCCT
                                                    1050
  Gly Asn Pro Phe Val Gly Val Thr Pro Trp Ala Asn Ala Tyr Tyr Ala
CTGAAGTTAGCAGCCTCGCTATTCCTAGCTTGACTGGAGCCATGGCCACT
                                                    1100
Ser Glu Val Ser Ser Leu Ala Ile Pro Ser Leu Thr Gly Ala Met Ala Thr
GCTGCAGCAGCTGTCGCAAAGGTTCCCTCTTTTATGTGGCTGTAGGTCCT
                                                    1150
 Ala Ala Ala Ala Val Ala Lys Val Pro Ser Phe Met Trp Leu
CCCGGAACCAAGGCAATCTGTTACTGAAGGCTCATCATTCACTGCAGAGA
                                                    1200
                                                      Asp
TACTCTTGACAAGACCCCTCTCATGGAGCAAACCTTGGCCGACATCCGCA
                                                    1250
  Thr Leu Asp Lys Thr Pro Leu Met Glu Gln Thr Leu Ala Asp Ile Arg
CCGCCAACAAGAATGGCGGTAACTATGCCGGACAGTTTGTGGTGATAGAC
                                                    1300
Thr Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Ile Asp
TTGCCGGATCGCGATTGCGCTGCCCTTGCCTCGAATGGCGAATACTCTAT
                                                    1350
 Leu Pro Asp Arg Asp Cys Ala Ala Leu Ala Ser Asn Gly Glu Tyr Ser Ile
TGCCGATGGTGGCGTCGCCAAATATAAGAACTATATCGACACCATTCGTC
                                                    1400
  Ala Asp Gly Gly Val Ala Lys Tyr Lys Asn Tyr Ile Asp Thr Ile Arg
AAATTGTCGTGGAATATTCCGATATCCGGACCCTCCTGGTTATTGGTATG
                                                    1450
Gln Ile Val Val Glu Tyr Ser Asp Ile Arg Thr Leu Leu Val Ile
AGTTTAAACACCTGCCTCCCCCCCCCCTTCCCTTCCTTTCCCGCCGGCAT
                                                    1500

CTTGTCGTTGTGCTAACTATTGTTCCCTCTTCCAGAGCCTGACTCTCTTG
                                                    1550
                                    Glu Pro Asp Ser Leu
CCAACCTGGTGACCAACCTCGGTACTCCAAAGTGTGCCAATGCTCAGTCA
                                                    1600
Ala Asn Leu Val Thr Asn Leu Gly Thr Pro Lys Cys Ala Asn Ala Gln Ser
GCCTACCTTGAGTGCATCAACTACGCCGTCACACAGCTGAACCTTCCAAA
                                                    1650
 Ala Tyr Leu Glu Cys Ile Asn Tyr Ala Val Thr Gln Leu Asn Leu Pro Asn
TGTTGCGATGTATTTGGACGCTGGCCATGCAGGATGGCTTGGCTGGCCGG
                                                    1700
  Val Ala Met Tyr Leu Asp Ala Gly His Ala Gly Trp Leu Gly Trp Pro
```

FIG._2B

RECTIFIED SHEET (RULE 91)

ISA/EP

```
CAAACCAAGACCCGGCCGCTCAGCTATTTGCAAATGTTTACAAGAATGCA                1750
Ala Asn Gln Asp Pro Ala Ala Gln Leu Phe Ala Asn Val Tyr Lys Asn Ala
TCGTCTCCGAGAGCTCTTCGCGGATTGGCAACCAATGTCGCCAACTACAA                1800
Ser Ser Pro Arg Ala Leu Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr Asn
CGGGTGGAACATTACCAGCCCCCCATCGTACACGCAAGGCAACGCTGTCT                1850
  Gly Trp Asn Ile Thr Ser Pro Pro Ser Tyr Thr Gln Gly Asn Ala Val
ACAACGAGAAGCTGTACATCCACGCTATTGGACCTCTTCTTGCCAATCAC                1900
Tyr Asn Glu Lys Leu Tyr Ile His Ala Ile Gly Pro Leu Leu Ala Asn His
GGCTGGTCCAACGCCTTCTTCATCACTGATCAAGGTCGATCGGGAAAGCA                1950
 Gly Trp Ser Asn Ala Phe Phe Ile Thr Asp Gln Gly Arg Ser Gly Lys Gln
GCCTACCGGACAGCAACAGTGGGGAGACTGGTGCAATGTGATCGGCACCG                2000
  Pro Thr Gly Gln Gln Gln Trp Gly Asp Trp Cys Asn Val Ile Gly Thr
GATTTGGTATTCGCCCATCCGCAAACACTGGGGACTCGTTGCTGGATTCG                2050
Gly Phe Gly Ile Arg Pro Ser Ala Asn Thr Gly Asp Ser Leu Leu Asp Ser
TTTGTCTGGGTCAAGCCAGGCGGCGAGTGTGACGGCACCAGCGACAGCAG                2100
Phe Val Trp Val Lys Pro Gly Gly Glu Cys Asp Gly Thr Ser Asp Ser Ser
TGCGCCACGATTTGACTCCCACTGTGCGCTCCCAGATGCCTTGCAACCGG                2150
  Ala Pro Arg Phe Asp Ser His Cys Ala Leu Pro Asp Ala Leu Gln Pro
CGCCTCAAGCTGGTGCTTGGTTCCAAGCCTACTTTGTGCAGCTTCTCACA                2200
Ala Pro Gln Ala Gly Ala Trp Phe Gln Ala Tyr Phe Val Gln Leu Leu Thr
AACGCAAACCCATCGTTCCTGTAAGGCTTTCGTGACCGGGCTTCAAACAA                2250
Asn Ala Asn Pro Ser Phe Leu •
TGATGTGCGATGGTGTGGTTCCCGGTTGGCGGAGTCTTTGTCTACTTTGG                2300
TTGTCTGTCGCAGGTCGGTAGACCGCAAATGAGCAACTGATGGATTGTTG                2350
CCAGCGATACTATAATTCACATGGATGGTCTTTGCGATCAGTAGCTAGTG                2400
AGAGAGAGAGAACATCTATCCACAATGTCGAGTGTCTATTAGACATACTC                2450
CGAGAATAAAGTCAACTGTGTCTGTGATCTAAAGATCGATTCGGCAGTCG                2500
AGTAGCGTATAACAACTCCGAGTACCAGCAAAAGCACGTCGTGACAGGAG                2550
CAGGCTTTGCCAACTGCGCAACCTTGCTTGAATGAGGATACACGGGGTGC                2600
```

FIG._2C

RECTIFIED SHEET (RULE 91)

ISA/EP

AACATGGCTGTACTGATCCATCGCAACCAAAATTTCTGTTTATAGATCAA 2650

GCTGGTAGATTCCAATTACTCCACCTCTTGCGCTTCTCCATGACATGTAA 2700

GTGCACGTAGGAAACCATACCCAAATTGCCTACAGCTGCGGAGCATGAGC 2750

CTATGGCGATCAGTCTGGTCATGTTAACCAGCCTGTGCTCTGACGTTAAT 2800

GCAGAATAGAAAGCCGCGGTTGCAATGCAAATGATGATGCCTTTGCAGAA 2850

ATGGCTTGCTCGCTGACTGATACCAGTAACAACTTTGCTTGGCCGTCTAG 2900

CGCTGTTGATTGTATTCATCACAACCTCGTCTCCCTCCTTTGGGTTGAGC 2950

TCTTTGGATGGCTTTCCAAACGTTAATAGCGCGTTTTTCTCCACAAAGTA 3000

TTCGTATGGACGCGCTTTTGGCTGTATTGCGTGAGCTACCAGCAGCCCAA 3050

TTGGCGAAGTCTTGAGCCGCACTCGCATAGAATAATTGATTGCGCATTTG 3100

ATGCGATTTTTGAGCGGCTGTTTCAGGCGACATTTCGCCGCCTTTATTTG 3150

CTCCATTATATCATCGATGGCATGTCCAATAGCCCGGTGATAGTCTTGTC 3200

GAATATGGCTGTCGTGGATAACCCATCGGCAGCAGATGATAATGATTCCG 3250

CAGCACAAGCTCGTATGTGGGTAGCAGAAGAACTGAGCGAGATCTTCGAG 3300

GGCGTAACTCTGCATATCCGATTGGCCTGCTGCCACATGTCATTTTGCTT 3350

CGGTTTCTTTTCTGTTGAGTTCTTGTATTTGGGTGAAAGTAACATGGTGT 3400

ATGACGAGAGACATTGGTGGTAAGAAAAAATTTCACCTCCTCTTAGTGCA 3450

GGACTGACTCTCAAAATCTATATGCAAATGTGTCGTGTAACACCCTTCGC 3500

ATGAGCGCTGACCGTACCCTACCATTTCGCCCCACTCATGATAGCAGAAG 3550

AGACATATTAATTCGGCAATGCTACGAAAGTCTGCAGGCTATGCTTAAAT 3600

AAACGCTTGCCACAGAAGCCGACAGTTTATTGTTACTACTTACTATACTG 3650

TATTATTGTTGCTCACATAAGGCGGTGAACCATTGGTTCACACGACGCCT 3700

GACGAGGTAAATTACTCTCTCGTAGGGCTGCCAAGGTAGGTCCCAACCCC 3750

GTATCCTCGGTCGAGGGTGCGAGGTTCTTTGGTCCTTCCCTCTTTGGTAA 3800

FIG._2D

AGCCCAGTAGCGTGTTTGAATCAGTTCACAATCTCTCCTAAACACAGTCC 3850

GACACTAGGTAGGTACGTTGTAATAGCAACTCAAACATGTAATTCGTTTC 3900

AAGGCAGGAACATTTTATAAACTTCCCTGCGATTTAATCAATAAAGATCC 3950

TAGTCCAATCGTATACTACCTACCTAGCTAAGGTAGGTAGGTAGTTCGTG 4000

GGAACCTGGTCGCTAATTCACGCAACCCACTTTGCGCTCTTCGCCTGGCC 4050

GTCGTTGAAGGTAAAGCAGTTGTACCCATCACCTAACTCAACCGACACCG 4100

TTGATCTGCTCAAGGCAGTTTTC 4123

FIG._2E

FIG._2A

FIG._2B

FIG._2C

FIG._2D

FIG._2E

FIG._2

```
TGTGTTGAAATCCAACTTATAAAGACAACAACCGCAAACTTTGTCTTGTG
                                                    50
CCATCAGATTGTTGCCAAGCACCGTCCCCCCCCCCTATCTTAGTCCTTCT
                                                   100
TGTTGTCCCAAAATGGCGCCCTCAGTTACACTGCCGTTGACCACGGCCAT
                                                   150
                  Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile
CCTGGCCATTGCCCGGCTCGTCGCCGCCCAGCAACCGGGTACCAGCACCC
                                                   200
 Leu Ala Ile Ala Arg Leu Val Ala Ala Gln Gln Pro Gly Thr Ser Thr
CCGAGGTCCATCCCAAGTTGACAACCTACAAGTGTACAAAGTCCGGGGGG
                                                   250
Pro Glu Val His Pro Lys Leu Thr Thr Tyr Lys Cys Thr Lys Ser Gly Gly
TGCGTGGCCCAGGACACCTCGGTGGTCCTTGACTGGAACTACCGCTGGAT
                                                   300
 Cys Val Ala Gln Asp Thr Ser Val Val Leu Asp Trp Asn Tyr Arg Trp Met
GCACGACGCAAACTACAACTCGTGCACCGTCAACGGCGGCGTCAACACCA
                                                   350
  His Asp Ala Asn Tyr Asn Ser Cys Thr Val Asn Gly Gly Val Asn Thr
CGCTCTGCCCTGACGAGGCGACCTGTGGCAAGAACTGCTTCATCGAGGGC
                                                   400
Thr Leu Cys Pro Asp Glu Ala Thr Cys Gly Lys Asn Cys Phe Ile Glu Gly
GTCGACTACGCCGCCTCGGGCGTCACGACCTCGGGCAGCAGCCTCACCAT
                                                   450
 Val Asp Tyr Ala Ala Ser Gly Val Thr Thr Ser Gly Ser Ser Leu Thr Met
GAACCAGTACATGCCCAGCAGCTCTGGCGGCTACAGCAGCGTCTCTCCTC
                                                   500
  Asn Gln Tyr Met Pro Ser Ser Ser Gly Gly Tyr Ser Ser Val Ser Pro
GGCTGTATCTCCTGGACTCTGACGGTGAGTACGTGATGCTGAAGCTCAAC
                                                   550
Arg Leu Tyr Leu Leu Asp Ser Asp Gly Glu Tyr Val Met Leu Lys Leu Asn
GGCCAGGAGCTGAGCTTCGACGTCGACCTCTCTGCTCTGCCGTGTGGAGA
                                                   600
 Gly Gln Glu Leu Ser Phe Asp Val Asp Leu Ser Ala Leu Pro Cys Gly Glu
GAACGGCTCGCTCTACCTGTCTCAGATGGACGAGAACGGGGGCGCCAACC
                                                   650
  Asn Gly Ser Leu Tyr Leu Ser Gln Met Asp Glu Asn Gly Gly Ala Asn
AGTATAACACGGCCGGTGCCAACTACGGGAGCGGCTACTGCGATGCTCAG
                                                   700
Gln Tyr Asn Thr Ala Gly Ala Asn Tyr Gly Ser Gly Tyr Cys Asp Ala Gln
TGCCCCGTCCAGACATGGAGGAACGGCACCCTCAACACTAGCCACCAGGG
                                                   750
 Cys Pro Val Gln Thr Trp Arg Asn Gly Thr Leu Asn Thr Ser His Gln Gly
CTTCTGCTGCAACGAGATGGATATCCTGGAGGGCAACTCGAGGGCGAATG
                                                   800
  Phe Cys Cys Asn Glu Met Asp Ile Leu Glu Gly Asn Ser Arg Ala Asn
```

FIG._3A

RECTIFIED SHEET (RULE 91)

ISA/EP

```
CCTTGACCCCTCACTCTTGCACGGCCACGGCCTGCGACTCTGCCGGTTGC
                                                    850
Ala Leu Thr Pro His Ser Cys Thr Ala Thr Ala Cys Asp Ser Ala Gly Cys
GGCTTCAACCCCTATGGCAGCGGCTACAAAAGGTGAGCCTGATGCCACTA
                                                    900
Gly Phe Asn Pro Tyr Gly Ser Gly Tyr Lys Ser
CTACCCCTTTCCTGGCGCTCTCGCGGTTTTCCATGCTGACATGGTTTTCC
                                                    950

AGCTACTACGGCCCCGGAGATACCGTTGACACCTCCAAGACCTTCACCAT
                                                   1000
   Tyr Tyr Gly Pro Gly Asp Thr Val Asp Thr Ser Lys Thr Phe Thr Ile
CATCACCCAGTTCAACACGGACAACGGCTCGCCCTCGGGCAACCTTGTGA
                                                   1050
  Ile Thr Gln Phe Asn Thr Asp Asn Gly Ser Pro Ser Gly Asn Leu Val
GCATCACCCGCAAGTACCAGCAAAACGGCGTCGACATCCCCAGCGCCCAG
                                                   1100
Ser Ile Thr Arg Lys Tyr Gln Gln Asn Gly Val Asp Ile Pro Ser Ala Gln
CCCGGCGGCGACACCATCTCGTCCTGCCCGTCCGCCTCAGCCTACGGCGG
                                                   1150
 Pro Gly Gly Asp Thr Ile Ser Ser Cys Pro Ser Ala Ser Ala Tyr Gly Gly
CCTCGCCACCATGGGCAAGGCCCTGAGCAGCGGCATGGTGCTCGTGTTCA
                                                   1200
  Leu Ala Thr Met Gly Lys Ala Leu Ser Ser Gly Met Val Leu Val Phe
GCATTTGGAACGACAACAGCCAGTACATGAACTGGCTCGACAGCGGCAAC
                                                   1250
Ser Ile Trp Asn Asp Asn Ser Gln Tyr Met Asn Trp Leu Asp Ser Gly Asn
GCCGGCCCCTGCAGCAGCACCGAGGGCAACCCATCCAACATCCTGGCCAA
                                                   1300
 Ala Gly Pro Cys Ser Ser Thr Glu Gly Asn Pro Ser Asn Ile Leu Ala Asn
CAACCCCAACACGCACGTCGTCTTCTCCAACATCCGCTGGGGAGACATTG
                                                   1350
  Asn Pro Asn Thr His Val Val Phe Ser Asn Ile Arg Trp Gly Asp Ile
GGTCTACTACGAACTCGACTGCGCCCCCGCCCCCGCCTGCGTCCAGCACG
                                                   1400
Gly Ser Thr Thr Asn Ser Thr Ala Pro Pro Pro Pro Pro Ala Ser Ser Thr
ACGTTTTCGACTACACCGAGGAGCTCGACGACTTCGAGCAGCCCGAGCTG
                                                   1450
 Thr Phe Ser Thr Thr Pro Arg Ser Ser Thr Thr Ser Ser Ser Pro Ser Cys
CACGCAGACTCACTGGGGGCAGTGCGGTGGCATTGGGTACAGCGGGTGCA
                                                   1500
  Thr Gln Thr His Trp Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Cys
AGACGTGCACGTCGGGCACTACGTGCCAGTATAGCAACGACTGTTCGTAT
                                                   1550
Lys Thr Cys Thr Ser Gly Thr Thr Cys Gln Tyr Ser Asn Asp
```

FIG._3B

RECTIFIED SHEET (RULE 91)

ISA/EP

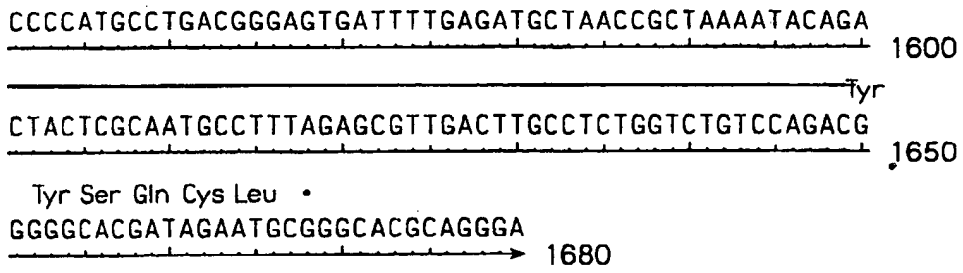

CCCCATGCCTGACGGGAGTGATTTTGAGATGCTAACCGCTAAAATACAGA
1600
Tyr
CTACTCGCAATGCCTTTAGAGCGTTGACTTGCCTCTGGTCTGTCCAGACG
1650
Tyr Ser Gln Cys Leu •
GGGGCACGATAGAATGCGGGCACGCAGGGA
1680

FIG._3C

FIG._3


FIG._3A
FIG._3B
FIG._3C

TGCCATTTCTGACCTGGATAGGTTTTCCTATGGTCATTCCTATAAGAGAC 50

ACGCTCTTTCGTCGGCCCGTAGATATCAGATTGGTATTCAGTCGCACAGA 100

CGAAGGTGAGTTGATCCTCCAACATGAGTTCTATGAGCCCCCCCCTTGCC 150

CCCCCCCGTTCACCTTGACCTGCAATGAGAATCCCACCTTTTACAAGAGC 200

ATCAAGAAGTATTAATGGCGCTGAATAGCCTCTGCTCGATAATATCTCCC 250

CGTCATCGACAATGAACAAGTCCGTGGCTCCATTGCTGCTTGCAGCGTCC 300

Met Asn Lys Ser Val Ala Pro Leu Leu Leu Ala Ala Ser
ATACTATATGGCGGCGCCGTCGCACAGCAGACTGTCTGGGGCCAGTGTGG 350

Ile Leu Tyr Gly Gly Ala Val Ala Gln Gln Thr Val Trp Gly Gln Cys Gly
AGGTATTGGTTGGAGCGGACCTACGAATTGTGCTCCTGGCTCAGCTTGTT 400

Gly Ile Gly Trp Ser Gly Pro Thr Asn Cys Ala Pro Gly Ser Ala Cys
CGACCCTCAATCCTTATTATGCGCAATGTATTCCGGGAGCCACTACTATC 450

Ser Thr Leu Asn Pro Tyr Tyr Ala Gln Cys Ile Pro Gly Ala Thr Thr Ile
ACCACTTCGACCCGGCCACCATCCGGTCCAACCACCACCACCAGGGCTAC 500

Thr Thr Ser Thr Arg Pro Pro Ser Gly Pro Thr Thr Thr Thr Arg Ala Thr
CTCAACAAGCTCATCAACTCCACCCACGAGCTCTGGGGTCCGATTTGCCG 550

Ser Thr Ser Ser Ser Thr Pro Pro Thr Ser Ser Gly Val Arg Phe Ala
GCGTTAACATCGCGGGTTTTGACTTTGGCTGTACCACAGAGTGAGTACCC 600

Gly Val Asn Ile Ala Gly Phe Asp Phe Gly Cys Thr Thr Asp
TTGTTTCCTGGTGTTGCTGGCTGGTTGGGCGGGTATACAGCGAAGCGGAC 650

GCAAGAACACCGCCGGTCCGCCACCATCAAGATGTGGGTGGTAAGCGGCG 700

GTGTTTTGTACAACTACCTGACAGCTCACTCAGGAAATGAGAATTAATGG 750

AAGTCTTGTTACAGTGGCACTTGCGTTACCTCGAAGGTTTATCCTCCGTT 800

Gly Thr Cys Val Thr Ser Lys Val Tyr Pro Pro Leu
GAAGAACTTCACCGGCTCAAACAACTACCCCGATGGCATCGGCCAGATGC 850

Lys Asn Phe Thr Gly Ser Asn Asn Tyr Pro Asp Gly Ile Gly Gln Met

FIG._4A

AGCACTTCGTCAACGAGGACGGGATGACTATTTTCCGCTTACCTGTCGGA 900

Gln His Phe Val Asn Glu Asp Gly Met Thr Ile Phe Arg Leu Pro Val Gly
TGGCAGTACCTCGTCAACAACAATTTGGGCGGCAATCTTGATTCCACGAG 950

Trp Gln Tyr Leu Val Asn Asn Asn Leu Gly Gly Asn Leu Asp Ser Thr Ser
CATTTCCAAGTATGATCAGCTTGTTCAGGGGTGCCTGTCTCTGGGCGCAT 1000

Ile Ser Lys Tyr Asp Gln Leu Val Gln Gly Cys Leu Ser Leu Gly Ala
ACTGCATCGTCGACATCCACAATTATGCTCGATGGAACGGTGGGATCATT 1050

Tyr Cys Ile Val Asp Ile His Asn Tyr Ala Arg Trp Asn Gly Gly Ile Ile
GGTCAGGGCGGCCCTACTAATGCTCAATTCACGAGCCTTTGGTCGCAGTT 1100

Gly Gln Gly Gly Pro Thr Asn Ala Gln Phe Thr Ser Leu Trp Ser Gln Leu
GGCATCAAAGTACGCATCTCAGTCGAGGGTGTGGTTCGGCATCATGAATG 1150

Ala Ser Lys Tyr Ala Ser Gln Ser Arg Val Trp Phe Gly Ile Met Asn
AGCCCCACGACGTGAACATCAACACCTGGGCTGCCACGGTCCAAGAGGTT 1200

Glu Pro His Asp Val Asn Ile Asn Thr Trp Ala Ala Thr Val Gln Glu Val
GTAACCGCAATCCGCAACGCTGGTGCTACGTCGCAATTCATCTCTTTGCC 1250

Val Thr Ala Ile Arg Asn Ala Gly Ala Thr Ser Gln Phe Ile Ser Leu Pro
TGGAAATGATTGGCAATCTGCTGGGGCTTTCATATCCGATGGCAGTGCAG 1300

Gly Asn Asp Trp Gln Ser Ala Gly Ala Phe Ile Ser Asp Gly Ser Ala
CCGCCCTGTCTCAAGTCACGAACCCGGATGGGTCAACAACGAATCTGATT 1350

Ala Ala Leu Ser Gln Val Thr Asn Pro Asp Gly Ser Thr Thr Asn Leu Ile
TTTGACGTGCACAAATACTTGGACTCAGACAACTCCGGTACTCACGCCGA 1400

Phe Asp Val His Lys Tyr Leu Asp Ser Asp Asn Ser Gly Thr His Ala Glu
ATGTACTACAAATAACATTGACGGCGCCTTTTCTCCGCTTGCCACTTGGC 1450

Cys Thr Thr Asn Asn Ile Asp Gly Ala Phe Ser Pro Leu Ala Thr Trp
TCCGACAGAACAATCGCCAGGCTATCCTGACAGAAACCGGTGGTGGCAAC 1500

Leu Arg Gln Asn Asn Arg Gln Ala Ile Leu Thr Glu Thr Gly Gly Gly Asn
GTTCAGTCCTGCATACAAGACATGTGCCAGCAAATCCAATATCTCAACCA 1550

Val Gln Ser Cys Ile Gln Asp Met Cys Gln Gln Ile Gln Tyr Leu Asn Gln
GAACTCAGATGTCTATCTTGGCTATGTTGGTTGGGGTGCCGGATCATTTG 1600

Asn Ser Asp Val Tyr Leu Gly Tyr Val Gly Trp Gly Ala Gly Ser Phe

FIG._4B

RECTIFIED SHEET (RULE 91)

ISA/EP

ATAGCACGTATGTCCTGACGGAAACACCGACTAGCAGTGGTAACTCATGG 1650

Asp Ser Thr Tyr Val Leu Thr Glu Thr Pro Thr Ser Ser Gly Asn Ser Trp

ACGGACACATCCTTGGTCAGCTCGTGTCTCGCAAGAAAGTAGCACTCTGA 1700

Thr Asp Thr Ser Leu Val Ser Ser Cys Leu Ala Arg Lys •

GCTGAATGCAGAAGCCTCGCCAACGTTTGTATCTCGCTATCAAACATAGT 1750

AGCTACTCTATGAGGCTGTCTGTTCTCGATTTCAGCTTTATATAGTTTCA 1800

TCAAACAGTACATATTCCCTCTGTGGCCACGCAAAAAAAAAAAAAAAAA 1849

FIG._4C

FIG._4

FIG._4A

FIG._4B

FIG._4C

GGGTGGTCTGGATGAAACGTCTTGGCCAAATCGTGATCGATTGATACTCG 50

CATCTATAAGATGGCACAGATCGACTCTTGATTCACAGACATCCGTCAGC 100

CCTCAAGCCGTTTGCAAGTCCACAAACACAAGCACAAGCATAGCGTCGCA 150

ATGAAGTTCCTTCAAGTCCTCCCTGCCCTCATACCGGCCGCCCTGGCCCA 200

Met Lys Phe Leu Gln Val Leu Pro Ala Leu Ile Pro Ala Ala Leu Ala Gln

AACCAGCTGTGACCAGTGGGCAACCTTCACTGGCAACGGCTACACAGTCA 250

Thr Ser Cys Asp Gln Trp Ala Thr Phe Thr Gly Asn Gly Tyr Thr Val

GCAACAACCTTTGGGGAGCATCAGCCGGCTCTGGATTTGGCTGCGTGACG 300

Ser Asn Asn Leu Trp Gly Ala Ser Ala Gly Ser Gly Phe Gly Cys Val Thr

GCGGTATCGCTCAGCGGCGGGGCCTCCTGGCACGCAGACTGGCAGTGGTC 350

Ala Val Ser Leu Ser Gly Gly Ala Ser Trp His Ala Asp Trp Gln Trp Ser

CGGCGGCCAGAACAACGTCAAGTCGTACCAGAACTCTCAGATTGCCATTC 400

Gly Gly Gln Asn Asn Val Lys Ser Tyr Gln Asn Ser Gln Ile Ala Ile

CCCAGAAGAGGACCGTCAACAGCATCAGCAGCATGCCCACCACTGCCAGC 450

Pro Gln Lys Arg Thr Val Asn Ser Ile Ser Ser Met Pro Thr Thr Ala Ser

TGGAGCTACAGCGGGAGCAACATCCGCGCTAATGTTGCGTATGACTTGTT 500

Trp Ser Tyr Ser Gly Ser Asn Ile Arg Ala Asn Val Ala Tyr Asp Leu Phe

CACCGCAGCCAACCCGAATCATGTCACGTACTCGGGAGACTACGAACTCA 550

Thr Ala Ala Asn Pro Asn His Val Thr Tyr Ser Gly Asp Tyr Glu Leu

TGATCTGGTAAGCCATAAGAAGTGACCCTCCTTGATAGTTTCGACTAACA 600

Met Ile Trp

FIG._5A

RECTIFIED SHEET (RULE 91)

ISA/EP

ACATGTCTTGAGGCTTGGCAAATACGGCGATATTGGGCCGATTGGGTCCT 650

Leu Gly Lys Tyr Gly Asp Ile Gly Pro Ile Gly Ser

CACAGGGAACAGTCAACGTCGGTGGCCAGAGCTGGACGCTCTACTATGGC 700

Ser Gln Gly Thr Val Asn Val Gly Gly Gln Ser Trp Thr Leu Tyr Tyr Gly

TACAACGGAGCCATGCAAGTCTATTCCTTTGTGGCCCAGACCAACACTAC 750

Tyr Asn Gly Ala Met Gln Val Tyr Ser Phe Val Ala Gln Thr Asn Thr Thr

CAACTACAGCGGAGATGTCAAGAACTTCTTCAATTATCTCCGAGACAATA 800

Asn Tyr Ser Gly Asp Val Lys Asn Phe Phe Asn Tyr Leu Arg Asp Asn

AAGGATACAACGCTGCAGGCCAATATGTTCTTAGTAAGTCACCCTCACTG 850

Lys Gly Tyr Asn Ala Ala Gly Gln Tyr Val Leu Ser

TGACTGGGCTGAGTTTGTTGCAACGTTTGCTAACAAAACCTTCGTATAGG 900

CTACCAATTTGGTACCGAGCCCTTCACGGGCAGTGGAACTCTGAACGTCG 950

Tyr Gln Phe Gly Thr Glu Pro Phe Thr Gly Ser Gly Thr Leu Asn Val

CATCCTGGACCGCATCTATCAACTAAAACCTGGAAACGTGAGATGTGGTG 1000

Ala Ser Trp Thr Ala Ser Ile Asn •

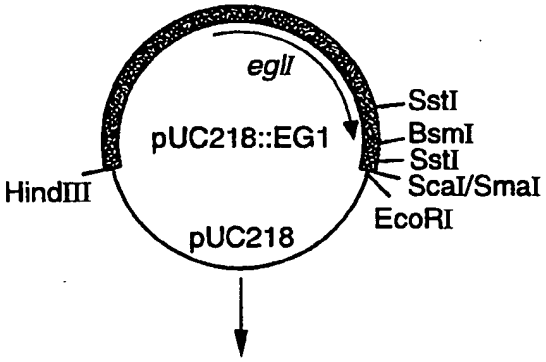
GGCATACGTTATTGAGCGAGGGAAAAAAAGCATTGGATCCATTGAAGATG 1050

FIG._5B

FIG._5A

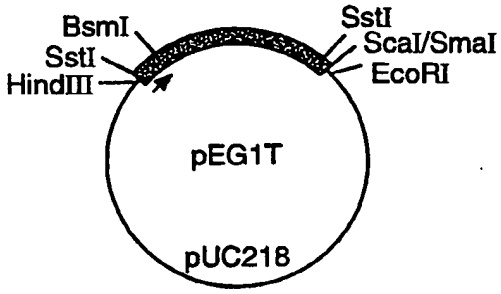
FIG._5

FIG._5B



- Digest with BsmI and EcoRI
- Isolate 300bp BsmI/EcoRI Fragment
- Digest pUC218 with SstI and EcoRI
- Ligate pUC218 SstI/EcoRI and BamI/EcoRI fragment with the following synthetic oligonucleotides (SEQ. ID NO:37)

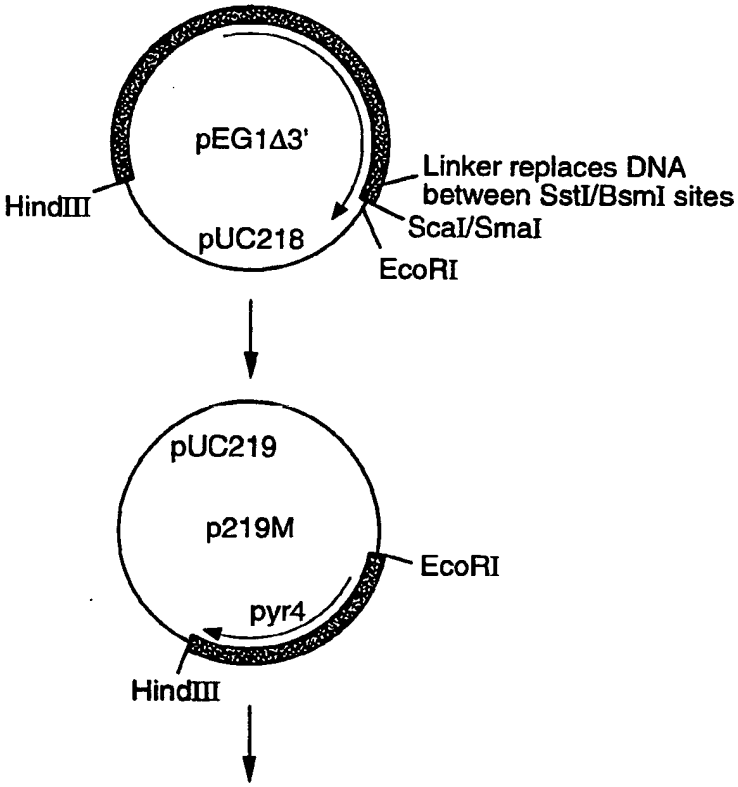
CGTAGAGCGTTGACTTGCCTGTGGTCTGTCCAGACGGGGGACGATAGAATGCG
TCGAGCATCTCGCAACTGAACGGACACCAGACAGGTCTGCCCCCTGCTATCTTAC
SstI BsmI



- Digest pEG1T with HindIII and BsmI and Isolate vector fragment
- Digest pUC218::EG1 with HindIII and SstI and Isolate 2.3 kb EG1 fragment
- Ligate pEG1T HindIII/BsmI and 2.3 Kb HindIII/SstI with the following synthetic oligonucleotides

CGTAGAGCGTTGACTTGCCTGTGGTCTGTCCAGACGGGGGACGATAGAATGCG
TCGAGCATCTCGCAACTGAACGGACACCAGACAGGTCTGCCCCCTGCTATCTTAC
SstI BsmI

FIG._6A



- Digest p219M with EcoRI and HindIII
- Isolate 1.6Kb EcoRI/HindIII *pyr4* gene fragment
- Digest pUC218 with EcoRI SstI and dephosphorylate the ends with calf alkaline phosphotase
- Isolate the HindIII/EcoRI EG1 fragment from pEG1Δ3'
- Ligate together pUC18 EcoRI, EcoRI/HindIII *pyr4* gene fragment and HindIII/EcoRI EG1 fragment

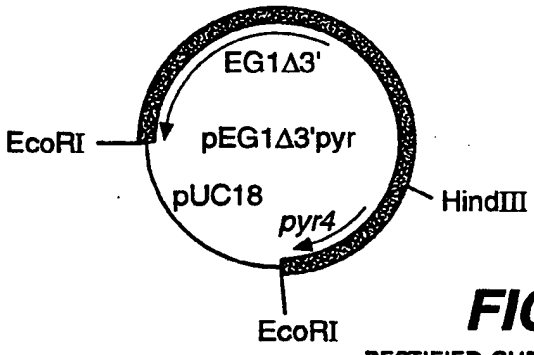


FIG._6A

FIG._6B

FIG._6

FIG._6B

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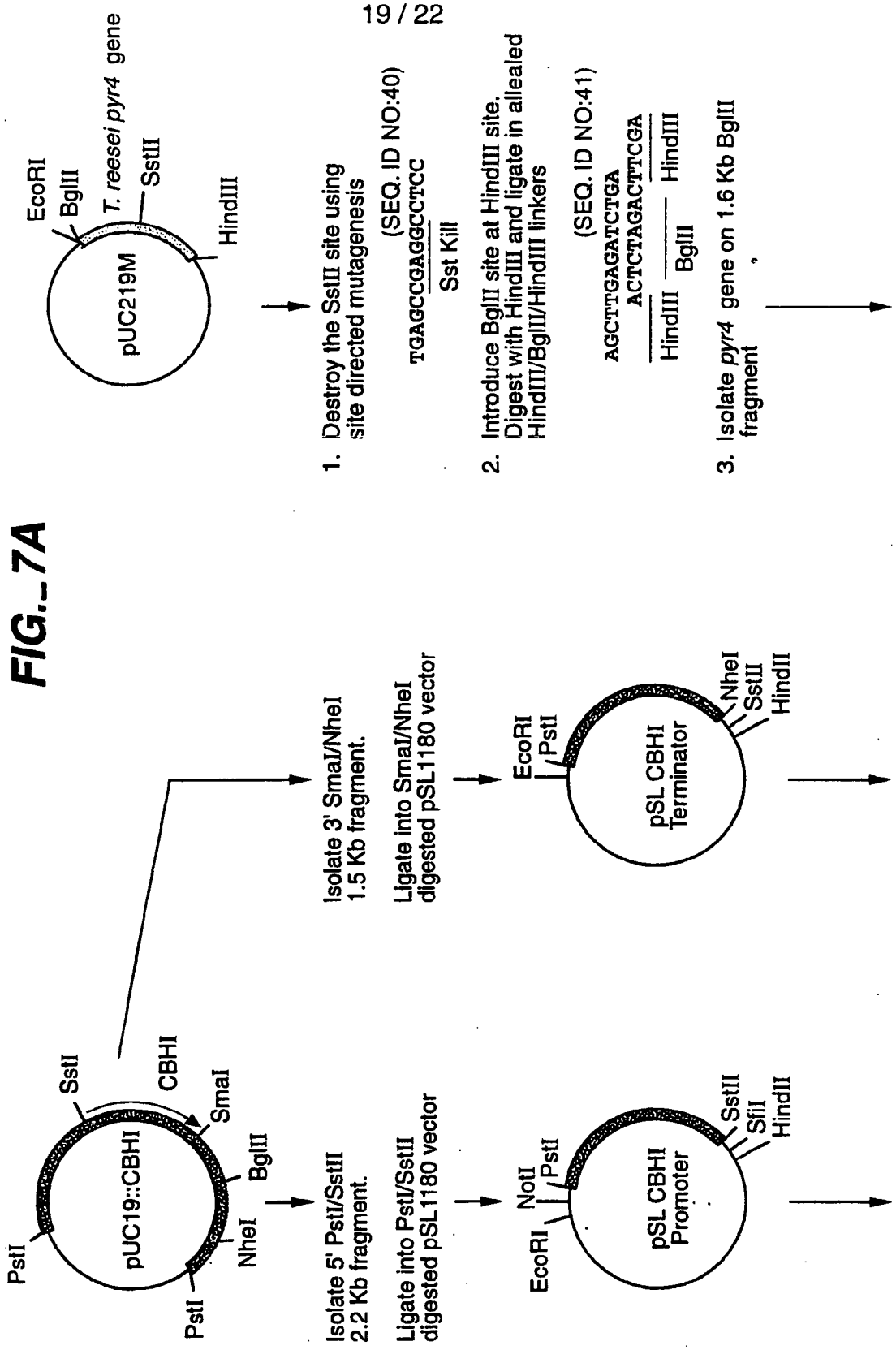
FIG._7A


EcoRI
BglII
T. reesei pyr4 gene
SstII
HindIII
pUC219M
1. Destroy the SstII site using site directed mutagenesis
(SEQ. ID NO:40)
TGAGCCGAGGCCTCC
Sst KiII
2. Introduce BglII site at HindIII site. Digest with HindIII and ligate in alleled HindIII/BglII/HindIII linkers
(SEQ. ID NO:41)
AGCTTGAGATCTGA
ACTCTAGACTTCGA
HindIII BglII HindIII
3. Isolate pyr4 gene on 1.6 Kb BglII fragment
PstI
SstI
CBHI
SmaI
BglII
NheI
PstI
pUC19::CBHI
Isolate 5' PstI/SstII 2.2 Kb fragment.
Ligate into PstI/SstII digested pSL1180 vector
Isolate 3' SmaI/NheI 1.5 Kb fragment.
Ligate into SmaI/NheI digested pSL1180 vector
EcoRI
NotI
PstI
pSL CBHI Promoter
SstII
SfiI
HindII
EcoRI
PstI
pSL CBHI Terminator
NheI
SstII
HindII

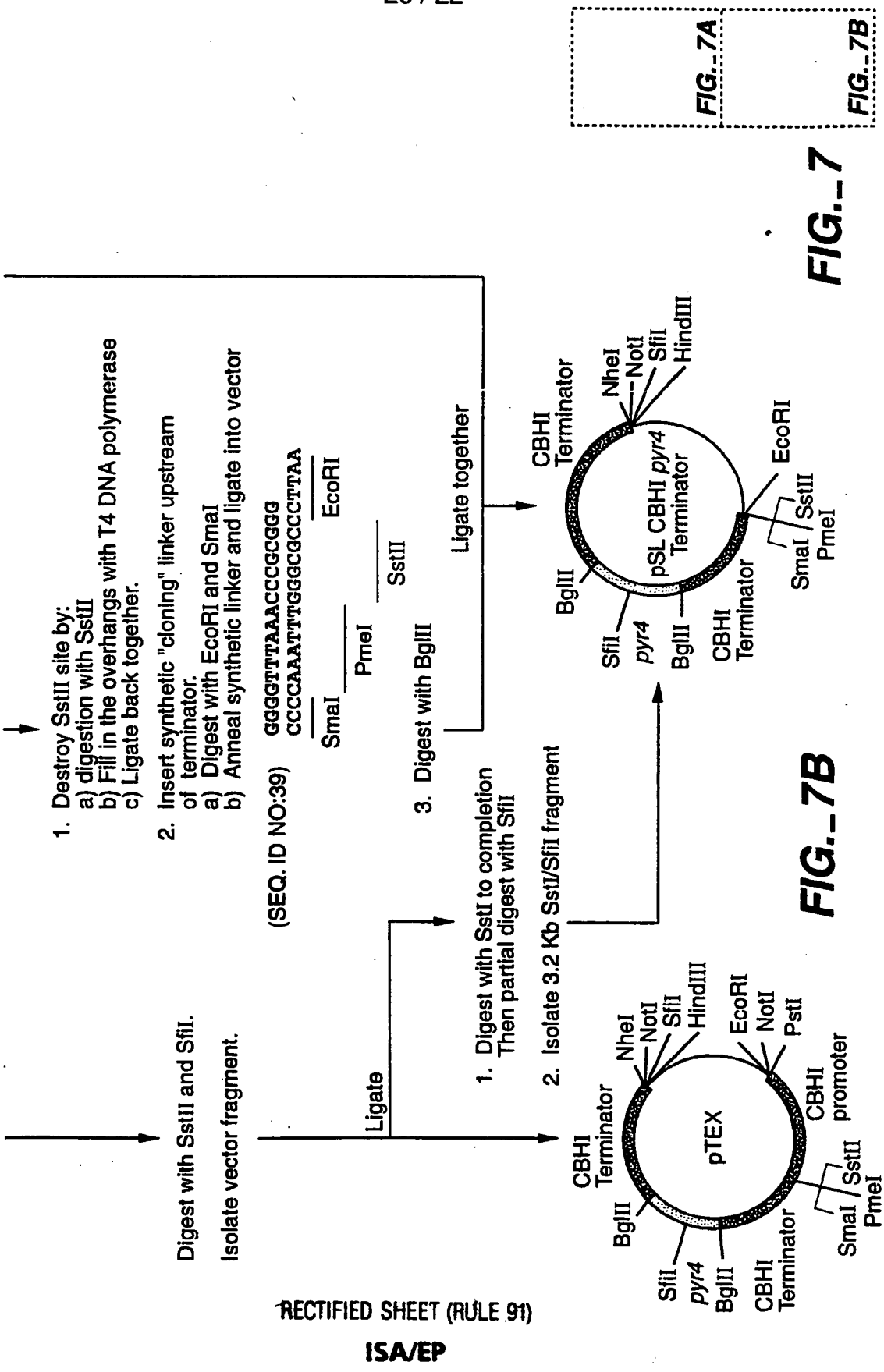

Digest with SstII and SfiI.
Isolate vector fragment.
1. Destroy SstII site by:
a) digestion with SstII
b) Fill in the overhangs with T4 DNA polymerase
c) Ligate back together.
2. Insert synthetic "cloning" linker upstream of terminator.
a) Digest with EcoRI and SmaI
b) Anneal synthetic linker and ligate into vector
(SEQ. ID NO:39) GGGGTTTAAACCCGCGGG
CCCCAAATTTGGGCGCCCTTAA
SmaI PmeI SstII EcoRI
3. Digest with BglII
Ligate together
Ligate
1. Digest with SstI to completion
Then partial digest with SfiI
2. Isolate 3.2 Kb SstI/SfiI fragment
pTEX
CBHI Terminator
NheI
NotI
SfiI
HindIII
EcoRI
NotI
PstI
CBHI promoter
SmaI
SstII
PmeI
BglII
SfiI
pyr4
BglII
CBHI Terminator
pSL CBHI pyr4 Terminator
CBHI Terminator
NheI
NotI
SfiI
HindIII
EcoRI
SmaI
SstII
PmeI
BglII
SfiI
pyr4
BglII
CBHI Terminator

FIG._7B

FIG._7

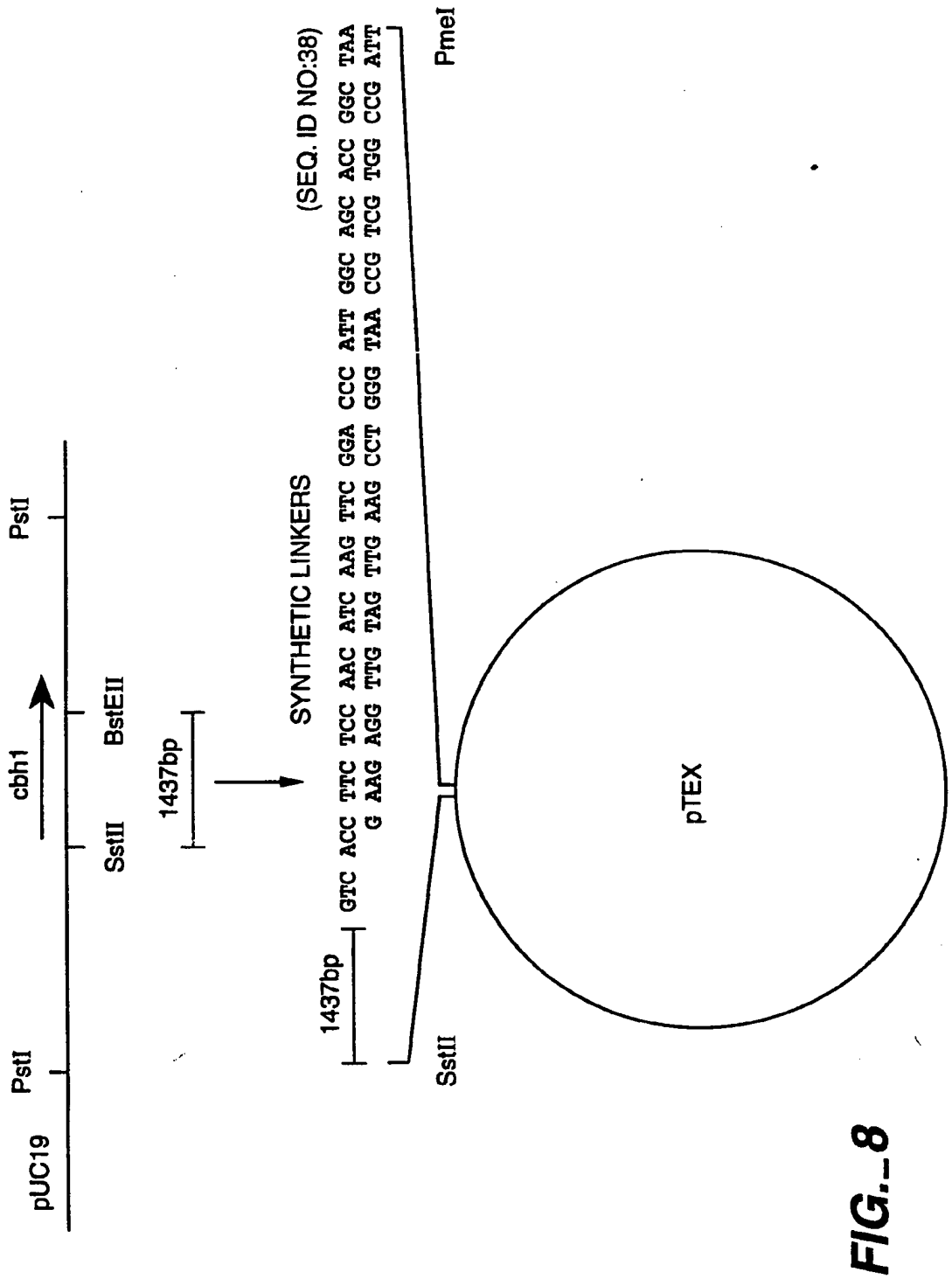

pUC19
PstI
cbh1
SstII
BstEII
PstI
1437bp
SYNTHETIC LINKERS
(SEQ. ID NO:38)
GTC ACC TTC TCC AAC ATC AAG TTC GGA CCC ATT GGC AGC ACC GGC TAA
G AAG AGG TTG TAG TTG AAG CCT GGG TAA CCG TCG TGG CCG ATT
PmeI
1437bp
SstII
pTEX

FIG._8

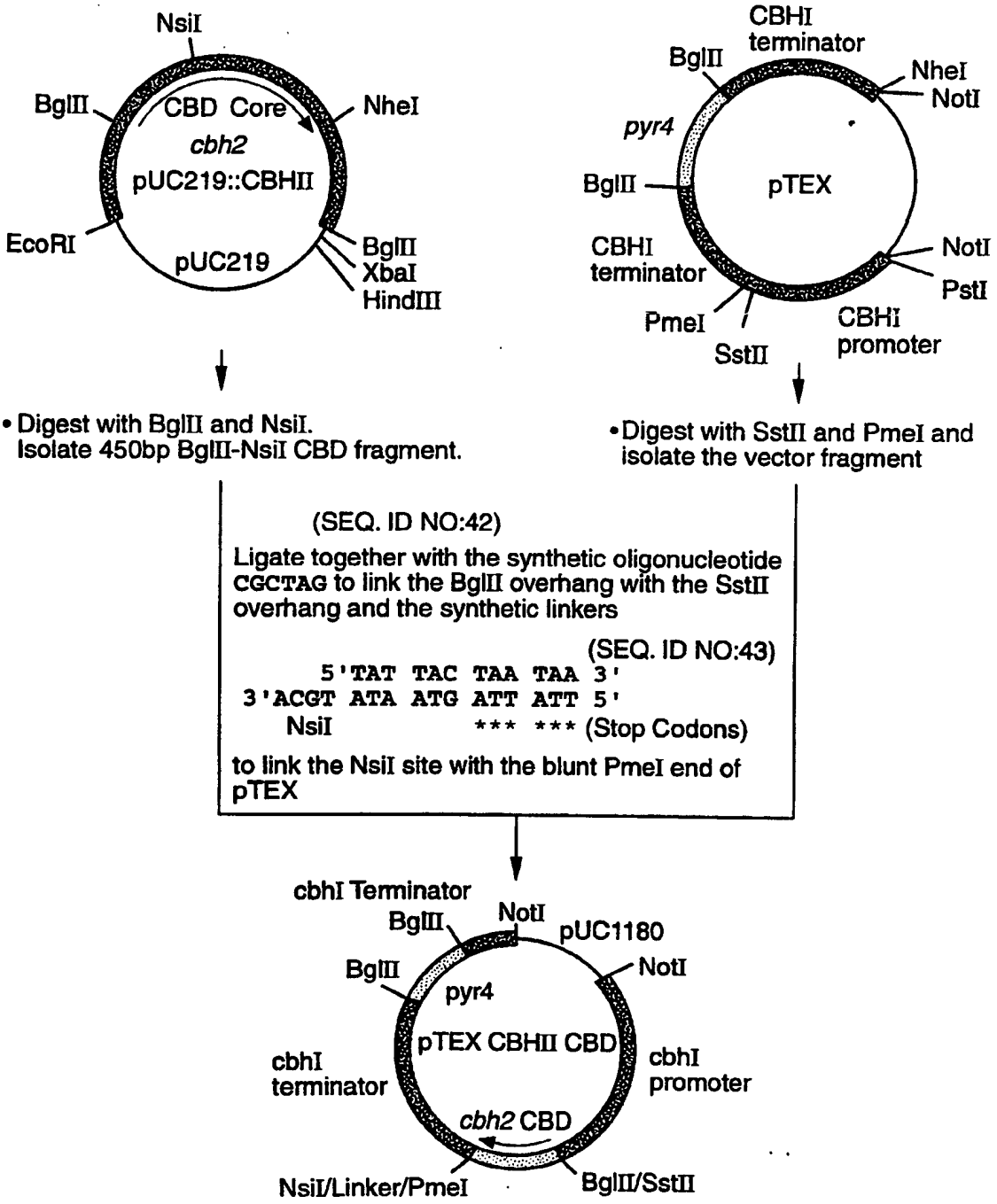


FIG._9

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INTERNATIONAL SEARCH REPORT

Interna ıl Application No
PCT/US 94/14163

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/80 C12N9/42 C12N15/52 C12N1/15 //(C12N1/15, C12R1:885)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EUROPEAN JORNAL OF BIOCHEMISTRY, vol. 200,no. 3, 15 September 1991 pages 643-649, SIRPA AHO ET AL. 'Monoclonal antibodies against core and cellulose-binding domains of Trichoderma reesei cellobiohydrolases I and II and endoglucanase I.' see the whole document ---	1-3,5-7
X	BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1087,no. 2, 1990 AMSTERDAM, pages 137-141, SIRPA AHO ET AL. 'The conserved terminal region of Trichodema reesei cellulases forms a strong antigenic epitope for polyclonal antibodies.' see the whole document ---	1-3

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 April 1995	20.04.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Delanghe, L

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FEBS LETTERS, vol. 291,no. 1, October 1991 AMSTERDAM NL, pages 45-49, SIRPA AHO 'Structural and functional analysis of Trichoderma reesei endoglucanase I expressed in yeast Saccharomyces cerevisiae.' see the whole document	1
X	FOUNDATION FOR BIOTECHNICAL AND INDUSTRIAL FERMENTATION RESEARCH, vol. 8, 1993 HANNOVER, pages 239-246, TIINA NAKARI ET AL. 'New Trichoderma promoters for production of hydrolytic enzymes on glucose medium.' (Trichoderma reesei cellulases and other hydrolases) see the whole document	1,67-70
A	WO,A,85 04672 (VALTION TEKNILLINEN TUTKIMUSKESKUS) 24 October 1985 see claims	1
A	EP,A,0 137 280 (CETUS CORPORATION) 17 April 1985 see claims	1
P,X	WO,A,94 07983 (GENENCOR INTERNATIONAL) 14 April 1994 see claims; figure 9B; example 13	66

1

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 94/14163

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8504672	24-10-85	CA-A-	1305931	04-08-92
		EP-A-	0214971	25-03-87
		EP-A-	0312121	19-04-89
		US-A-	5393670	28-02-95
		US-A-	4894338	16-01-90
EP-A-137280	17-04-85	AU-B-	589112	05-10-89
		AU-A-	3253084	07-03-85
		DE-A-	3485558	16-04-92
		JP-B-	6016709	09-03-94
		JP-A-	60149387	06-08-85
		JP-A-	7051071	28-02-95
WO-A-9407983	14-04-94	AU-B-	4924793	26-04-94